
UNIVERSITY
OF TASMANIA

School of Agricultural Science

# *Spirulina*: Dual-purpose lamb supplement - Breed and sex effects on productivity and product quality

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*"…settle mate. All you are is a farmer what can read good"*
**C.D. Banks**

# Declaration

This is to certify that:

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# Abstract

This thesis tested the effect of *Spirulina* supplementation and its interactions with sire breed, sex and nutritional plane on Australian dual-purpose lamb productivity and product quality. It was hypothesised that *Spirulina* supplementation would interact with sex, sire breed and plane of nutrition to affect wool and meat quality traits, haematological metabolites, body conformation, liveweight and feed intake indices of lamb productivity. Secondary objectives of assessing the independent effects of sire breed, sex and plane of nutrition on the fatty acid composition of lamb were also investigated.

A total of 48 lambs randomly allocated to treatment groups balanced by sire breed (Black Suffolk, Dorset, Merino, and White Suffolk), sex (ewes and wethers) and *Spirulina* supplementation levels (CONTROL – 0mL, LOW – 50mL, MEDIUM – 100mL, and HIGH – 200mL) was used. These lambs were allocated into two feeding trials with basal diets of differing planes of nutrition – low and high planes of nutrition which represented simulated-drought and pasture-fed diets respectively. In both trials, lambs were fed daily with 150 g barley grain and assigned *Spirulina* supplementation level as a 1:10 w/v water suspension using a drenching gun prior to being released for grazing on ryegrass pastures. Each feeding trial lasted for 9-weeks including a 3-week adjustment phase.

Data collected throughout the feeding trial included; weekly body conformation and liveweight measurements and feed intake; midside wool and blood samples; carcass

samples and dressed weight; and feed composition. Wool and meat quality traits, haematological metabolites, body conformation, liveweight and feed intake indices of productivity were analysed in SAS with *Spirulina* supplementation level, sire breed, sex, plane of nutrition and their second-order interactions used as fixed effects.

It was evident that *Spirulina* supplementation level, sire breed, sex and nutritional plane significantly affected productivity and product quality in Australian dual-purpose lambs, in that; 1) Medium and high *Spirulina* supplementation levels improved liveweight and growth during drought periods of low nutritional planes; 2) *Spirulina* supplementation affected physical wool quality traits only when interacting with sire breed; 3) As *Spirulina* supplementation level increased, so did gamma-glutamyl plasma concentrations and significant interactions of supplementation level with sex had an impact on glucose, Aspartate aminotransferase, and magnesium. Interactions of level of supplementation with sire breed influenced creatinine, gamma-glutamyl and albumin/globulin ratios; 4) *Spirulina* had no effect on intramuscular fat levels but reduced fat levels when the lambs were on a high nutritional plane; 5) High levels of *Spirulina* supplementation were associated with increase in subcutaneous fat melting point; and 6) *Spirulina* level did not reduce feed intake and had no detrimental effect on wool quality of lambs. Furthermore, regardless of *Spirulina* supplementation level, sex, sire breed and nutritional plane influenced fatty acid profile as an indicator of meat quality. Consequently, the hypothesis was accepted.

As an outcome of this thesis, Australian dual-purpose lamb operations can apply *Spirulina* supplementation to best optimise product quality and productivity. It permits informed decisions when tailoring animal management systems and responding to dietary shifts. The impacts of sire breed, sex and plane of nutrition on lamb product quality and productivity contributes to the existing knowledge of supplementing ruminants with microalgae. This thesis demonstrates that *Spirulina* is a beneficial and useful protein-rich supplement for Australian dual-purpose lamb operations.

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# List of Abbreviations

**∑**, Sum of
**Δ**, Change in
**2D**, Two dimensional
**AA**, Amino acids
**A/G Ratio**, Albumin/globulin ratio
**ADF**, Acid detergent fibre
**ADG**, Average daily liveweight / bodyweight gain
**ALA**, Alpha-linolenic acid (18:3n-3)
**ARA**, Arachidonic acid (20:4n-6)
**AST**, Aspartate aminotransferase
**ATLAS**, Automatic tester for length and strength
**AWTA**, Australian Wool Testing Authority
**AWET**, Australian Wool Education Trust
**BCS**, Body condition score
**BHB**, Beta-hydroxybutyrate
**BL**, Body length
**BWT**, Liveweight / bodyweight
**CF**, Comfort factor
**CG**, Chest girth
**CK**, Creatine kinase
**CLA**, Conjugated linoleic acid
**CSIRO**, Commonwealth Scientific and Industrial Research Organisation
**CSL**, Central Science Laboratory
**CURV**, Fibre curvature
**CV**, Coefficient of variation of fibre diameter
**DFI**, Daily feed intake
**DHA**, Docosahexaenoic acid (22:6)
**DM**, Dry matter
**DNA**, Deoxyribonucleic acid
**DPA**, Docosapentaenoic acid (22:5)
**DPIPWE**, Department of Primary Industries, Parks, Water and Environment
**EBV**, Estimated breeding value
**EE**, Ether extract
**EPA**, Eicosapentaenoic acid (20:5n-3)
**EU**, European Union
**$F_1$**, First cross
**FA**, Fatty acids
**FCR**, Feed conversion ratio
**$FCR_{met}$**, Metabolic FCR
**FD**, Mean fibre diameter
**FDP**, Fibre diameter profile
**FMP**, Subcutaneous fat melting point
**GGT**, Gamma-glutamyl transferase
**GLDH**, Glutamate dehydrogenase
**IFP**, Intermediate-filament keratin proteins

**IMF**, Intramuscular fat percentage
**IOM**, Indigestible organic matter
**KAP**, Keratin associated proteins
**LA**, Linoleic acid (18:2n-6)
**LCn-3**, Long chain n-3
**ME**, Metabolisable energy
**MUFA**, Monounsaturated FA
**n-3**, Omega-3 PUFA
**n-6**, Omega-6 PUFA
**NDF**, Neutral detergent fibre
**NDFn**, Nitrogen free NDF
**NEFA**, Non esterfied fatty acids
**NFC**, Non fibrous carbohydrate
**OFDA**, Optical fibre diameter analysis
**PF**, Prickle factor
**PUFA**, Polyunsaturated FA
**RFI**, Residual feed intake model
**RLG**, Residual liveweight gain model
**SAS,** Statistical Analysis Software
**SBR**, Staple breakage region
**SD**, Standard deviation of fibre diameter
**SDFI**, Standardised daily feed intake model
**SF**, Spinning fineness
**SFA**, Saturated FA
**SGR**, Specific growth rate
**SIFAN**, Single fibre analyser
**SL**, Staple length
**SS**, Staple strength
**TIA**, Tasmanian Institute of Agriculture
**UFA**, Unsaturated FA
**USA**, United States of America
**UTAS**, University of Tasmania
**WH**, Wither height
**YIELD**, Clean fleece percentage yield

# List of Publications

During the course of this study a number of publications and public presentations have been made which are based on the work presented in this thesis. They are listed below for future reference.

**Journal Articles**

Holman, BWB, Kashani, A, Nichols, PD & Malau-Aduli, AEO 2013, 'Sire breed and sex variations in the fatty acid composition of heart, kidney, liver and muscle tissues of Australian lambs', *Meat Science*, (Submitted).

Holman, BWB, Kashani, A, Malau-Aduli, AEO & Nichols, PD 2013, 'Effect of nutritional plane on the fatty acid profiles of heart, kidney, liver, adipose and muscle tissues of Australian dual-purpose lambs', *Animal*, (Submitted).

Holman, BWB, Kashani, A & Malau-Aduli, AEO 2013, 'Effect of *Spirulina* (*Arthrospira platensis*) supplementation on wool quality in genetically divergent dual-purpose Merino lambs under typical pasture-based management and stimulated-drought', *Animal*, (Submitted).

Holman, BWB, Flakemore, AR, Kashani, A & Malau-Aduli, AEO 2013, 'Influence of *Spirulina* supplementation, sire breed, sex and nutritional plane on intramuscular fat percentage and fat melting point in crossbred and purebred Merino lambs', *Animal Feed Science and Technology*, (Submitted).

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Holman, BWB & Malau-Aduli, AEO 2013, 'Effect of *Spirulina* supplementation on plasma metabolites in crossbred and purebred Australian Merino lambs', *Journal of Applied Animal Research*, (Submitted).

Holman, BWB, Kashani, A & Malau-Aduli, AEO 2013, 'Modelling the feed intake of purebred and crossbred Australian Merino lambs supplemented with *Spirulina*', *Journal of Animal Science and Biotechnology*, (Submitted).

Holman, BWB, Kashani, A & Malau-Aduli, AEO 2013, 'Wool quality traits of grazing Australian purebred and crossbred Merino lambs orally drenched with *Spirulina* (Arthrospira platensis)', *American Journal of Experimental Agriculture*, (Submitted).

Holman, BWB, Kashani, A & Malau-Aduli, AEO 2012, 'Growth and body conformation responses of genetically divergent Australian sheep to *Spirulina* (*Arthrospira platensis*) supplementation', *American Journal of Experimental Agriculture,* vol. 2**,** pp. 160-173.

**Review Papers**

Holman, BWB & Malau-Aduli, AEO 2012, '*Spirulina* as a livestock supplement and animal feed', *Journal of Animal Physiology and Animal Nutrition,* (In Press).

Holman, BWB & Malau-Aduli, AEO 2012, 'A review of sheep wool quality traits', *Annual Review and Research in Biology,* vol. 2**,** pp. 1-14.

**Conferences and Presentations**

Holman, BWB, Kashani, A & Malau-Aduli, AEO 2013, 'Relationships between wool quality, body conformation and liveweight measurements in genetically divergent sheep supplemented with *Spirulina*', in *Proceedings of the 11$^{th}$ World Conference on Animal Production*, 15-20$^{th}$ October, Beijing International Convention Centre, Beijing, China.

Malau-Aduli AEO, Flakemore, AR, Holman, BWB, Kashani, A & Lane, P 2013, '*Arthrospira platensis*: A novel feed supplement improves meat eating quality of Australian lamb', in *Proceedings of the 11th World Conference on Animal Production*, 15-20th October, Beijing International Convention Centre, Beijing, China.

Holman, BWB, Kashani, A, Nichols, PD & Malau-Aduli, AEO 2011, '*Spirulina* supplementation does not compromise wool quality', in *Proceedings of Graduate Research and Sharing Excellence in Research (SEiR) conference*, 1-2 September, Hobart, Tasmania.

Holman, BWB 2012, 'Effect of *Spirulina* supplementation on $F_1$ crossbred and purebred Merino lamb productivity – an introductory PhD seminar', in *School of Agricultural Science/Tasmanian Institute of Agriculture Seminar Series*'. 24th February, Sandy Bay, Tasmania.

Holman, BWB 2013, 'Effect of *Spirulina* supplementation on $F_1$ crossbred and purebred Merino lamb productivity – final PhD seminar', in *School of Agricultural Science/Tasmanian Institute of Agriculture Seminar Series*'. 7th June, Sandy Bay, Tasmania.

# Introduction

*Spirulina* (*Arthrospira platensis*) is an edible cyanobacterium which ancient humans would consume. However, only in relatively recent decades has *Spirulina* been identified as an apt livestock feed and supplement. This is due to its protein-rich composition, in conjunction with its many essential vitamins, minerals, amino acids, fatty acids and carotenoids. *Spirulina* has already been trialled in feed rations for pigs, rabbits, chickens and cattle by producers and researchers alike. These have found *Spirulina* supplementation to affect both productivity and product quality traits unique to these animal species. Yet, to the best of our knowledge, understanding regarding the effect of *Spirulina* supplementation in lamb production settings remains scarce.

Australian lamb producers have actively adopted dual-purpose production systems irrespective of environmental production region or basal feed availability, nutritional plane or type – being both typical pasture-based grazing and supplemented drought basal diets. These generally mate terminal meat-type sire breeds to a core flock of purebred Merino ewes in order to combine desirable meat and wool characteristics into any progeny. This allows Australian dual-purpose lamb producers to maintain interests in both the comparatively lesser valued wool and high valued meat markets simultaneously and using a single flock. The disparity between these markets returns to producers has prompted the many lambs to be supplemented with protein-rich feed types to boost liveweight growth and its associated profitability. Consequently, dual-purpose lamb producer's bifocal market share relies heavily on genetic and dietary supplementation management strategies to optimise and balance all productivity and product quality facets.

Dual-purpose lamb productivity and product quality can be objectively defined by using and categorising as:

- **Wool** is a fibrous product predominated by sulphur-rich keratin proteins. These confer wool's unique durability, rigidity, strength and processing performance characteristics to each fibre. Wool quality is often quantified by its physical wool traits, which permit accurate price and end use discrimination, ideally prior to processing or purchase.
- **Meat**, or muscle tissue, is the primary consumable lamb product with its heightened yield highly sorted. Several lamb organs, including kidney, liver and heart, are also eaten albeit dependent on ethic/cultural backgrounds. Productivity can be monitored using animal liveweights, body conformation and growth rates. Haematological metabolites can also contribute to productivity insights, as well as into animal health and response to nutritional shifts. Similarly, understanding feed intake and its conversion to meat provides productivity information. In combination with productivity, sensory and nutritional meat qualities also contribute to meat value. Sensory quality refers to consumer stimulatory response upon maceration whereas nutritional quality refers to inferred health benefits. Fortunately, both these aspects can be assessed with fatty acid content and composition scrutiny.

Identified by a comprehensive investigation of current published literature, is a death of knowledge regarding the effect of *Spirulina* supplementation on product quality and productivity in lambs. This posed several questions:

1. How will *Spirulina* supplementation affect lamb liveweight, body conformation and growth?
2. Will *Spirulina* influence wool and/or meat quality traits in supplemented lambs?
3. Can lamb health, feed intake and nutrient partitioning priorities be managed through *Spirulina* supplementation?
4. What will the effects of lamb sire breed and sex have on responses to *Spirulina* supplementation?

5. At which level and basal diet/plane of nutrition will *Spirulina* supplementations effect on lamb productivity and product quality be most effective?
6. Would lamb sire breed, sex and basal diet/plane of nutrition independently shift analysed productivity and product quality traits?

Therefore, the aim of this thesis was to assess the effect of *Spirulina* supplementation and its interactions with sire breed, sex and plane of nutrition on Australian dual-purpure lamb productivity and product quality. It was hypothesised that *Spirulina* supplementation would both independently and interact with sire breed, sex and plane of nutrition to alter lamb product quality and productivity. This initial aim was undertaken in parallel to a secondary objective of testing the independent effects of sire breed, sex, plane of nutrition and their interactions on these same parameters.

This thesis addresses these objectives as individual chapters dedicated to assessing subordinate hypotheses and objectives which together contribute to successful answering of the overarching objective and hypothesis and preliminary queries. The topics covered in each chapter are stated below.

**Chapter One** explores existing published literature to formulate a brief and comprehensive literature review discussing Australia's wool and meat industry, product quality, productivity, quantification methods, responses to genetic and nutritional management, and *Spirulina* as a livestock supplement.

**Chapter Two** evaluates the effect of *Spirulina* supplementation level and its interactions with sire breed, sex, and plane of nutrition on crossbred and purebred Merino lambs' physical wool quality trait with the hypothesis that an incremental decline in wool quality will be observed with increased *Spirulina* supplementation level over the feeding trial duration as per lamb sire breed, sex or plane of nutrition.

**Chapter Three** tests the hypothesis that *Spirulina* supplementation level will not compromise physical wool traits in lambs with high nutritional plane by investigating the effect of *Spirulina* supplementation level on wool characteristics of grazing crossbred and purebred Merino weaned lambs, and their interactions with sire breed and sex.

**Chapter Four** explores the hypothesis that liveweight, body conformation and growth has a positive association with *Spirulina* supplementation level, which is influenced by nutritional plane, sex and sire breed, by assessing the effect of *Spirulina* supplementation level and its interactions with plane of nutrition, sire breed and sex on liveweight, body conformation and growth changes over the duration of a feeding trial in Australian dual-purpose lambs.

**Chapter Five** investigates the hypothesis that supplementation with *Spirulina* will increase liveweight, growth and body conformation traits with significant interactions between sire breed and sex by evaluating the effect of varying levels of *Spirulina* supplementation, sire breed and gender on liveweight and body conformation traits.

**Chapter Six** tests the hypothesis that *Spirulina* supplementation will not be detrimental to the health and productivity of crossbred and purebred Merino lambs as indicated by haematological profiles, but interactions between *Spirulina* supplementation level, sire breed and sex will drive this variation, with the objective to study the effect of supplementing purebred and crossbred Merino lambs with *Spirulina* on plasma metabolite concentrations and interactions with sire breed and sex when fed basal diet of ryegrass pasture and barley grains (high plane of nutrition).

**Chapter Seven** evaluates the effects of *Spirulina* supplementation, sire breed, sex and their second-order interactions on voluntary feed intake in Australian dual-purpose lambs fed a basal diet of lucerne hay and barley grains (low plane of nutrition).

**Chapter Eight** analyses the effect of *Spirulina* supplementation on intramuscular fat percentage and subcutaneous adipose saturated/unsaturated fatty acid content using fat melting points with the hypothesis that *Spirulina* supplementation level would independently and interact with sex, sire breed and plane of nutrition increase intramuscular fat percentage and unsaturated fatty acid content.

**Chapter Nine** describes the effect of plane of nutrition and its interactions with sire breed and sex on the fatty acid content and composition of lamb subcutaneous adipose, Longissimus dorsi muscle, kidney, liver and heart tissues in Australian dual-purpose lambs.

**Chapter Ten** investigates the effects of sire breed and sex on subcutaneous adipose, muscle, kidney, heart and liver tissues fatty acid composition in dual-purpose Australian crossbred and purebred Merino lambs to test the hypothesis that tissue fatty acid composition varied dependent on lamb sire breed and sex.

**Chapter Eleven** presents a holistic and integrated summary of thesis findings and their relation with initial major hypothesis and objectives, while identifying paucities requiring future investigations.

**Appendices** contain supplementary articles, presentations and declarations which complement this thesis yet were excluded from chapters due to format restrictions and/or direct relevance.

It should be noted while reading this thesis that it was constructed as per the University of Tasmania, School of Agricultural Science and Tasmanian Institute of Agriculture PhD Thesis by Publication format and requirements. Nevertheless, efforts have been made towards 'streamlining' and standardising of chapters. The UTAS adapted Harvard referencing style was used throughout this thesis.

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Chapter 1

# Literature review

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**ABSTRACT**

Australian dual-purpose lamb producers have both meat and wool market interests which are most profitable when product quality and productivity are maximised. Wool quality can be objectively monitored in terms of physical wool traits and proteomic profile, meat quality is assessed by evaluating intramuscular fat content, fat melting point and fatty acid composition, and lamb productive performance is quantified as a measure of liveweight, body conformation, growth and haematological metabolites. These parameters provide useful physiological and metabolic insights into animal response to genetic and nutritional management approaches typical of Australian producers. Genetic effects include sire breed selection, lamb sex and prioritisation based on Estimated Breeding Values (EBV) within a flock. Nutrition effects include supplementation as affected by type, level and basal diet interactions and ruminal digestion. An emerging protein-rich supplement of interest is *Spirulina* (*Arthrospira platensis*), having been trialled in chicken, pig, rabbit and ruminant diets. Highlighted in this review is the relative paucity in present knowledge to the effect of *Spirulina* supplementation to dual-purpose lambs and wool quality and proteome, body conformation and growth, haematological metabolites and meat quality responses. The primary objective of this review was to define and explore these aforementioned quality traits,

quantification approaches, genetic and nutritional effects of *Spirulina* supplementation in sheep and associations with haematological metabolites in order to identify knowledge gaps and proffer research solution to fill in the missing links.

(**Keywords**: sheep industry, wool, meat, productivity, product quality, *Spirulina*, genetics, nutrition, proteomics)

**The Sheep Industry**

A common saying is that "Australia was born on a sheep's back'', but times and old dependencies change. Over recent years, the sheep industry has intensified its production systems and maintained a concerted focus on its two primary market interests – wool and meat.

*Wool market*

Since the 1991 collapse of the national Australian wool pricing scheme, wool prices have declined to historic lows (Kopke *et al.*, 2008). Several contributory factors have been identified. They include:

1. Increased competition from synthetic and other natural fibre types (Valera *et al.*, 2009).
2. Limited expansion potential within current markets (Rogers, 2006).
3. Heightened production costs (Rowe, 2010).
4. Comparatively strong Australian dollar value (Gibbon and, Nolan, 2011).
5. Climatic events in key production regions (Harle *et al.*, 2007).

Consequently, Australia's national flock numbers have dwindled relative to historic trends. However, wool remains a primary market for sheep producers with great national and economic importance (Bardsley, 1994; Valera et al., 2009) because wool outperforms other fibres regarding insulation, moisture absorption, anti-static and fire-resistant properties (Rogers, 2006). Furthermore, Australia maintains a lion's

share of global superfine wool produced; over 90% of total production (Swan, 2010). This has been aided by the wool specialist Merino breeds contributing approximately 88% of the national wool clip (Hatcher *et al.*, 2010; Swan, 2010) and to Australia's global reputation for high quality wool.

*Meat market*

Australian lamb meat prices experienced resurgence during the late 2000s, reaching historically high levels. This was prompted by strengthened export demands from traditional USA, EU and new Asian markets experiencing economic expansion (Hopkins *et al.*, 2007b; Martin and, Phillips, 2011). Furthermore, as sheep meat constitutes approximately 3% of global meat production (Rowe, 2010), this market has vast growth potential.

As the Australian lamb meat market has become increasingly more export-oriented, it is exposed to increased vulnerabilities including among others, the following:

- Climatic events affecting supply and delivery (Martin and, Phillips, 2011).
- Economic and animal welfare concerns in export markets.
- Reduced land and feed resource availability (Smith *et al.*, 2010).
- Increased production costs and comparatively strong Australian dollar value.

Fortunately, domestic consumption is strong because Australians consume 11 kg of sheep meat products annually on the average (McLeod *et al.*, 2010) and only 45% of total lamb meat produced is exported (Pethick *et al.*, 2010a).

*Dual-purpose production systems*

Dual-purpose production systems have shared wool and meat market interests. This allows commodity diversification to provide a buffer against individual market volatilities (Kopke et al., 2008), and greater land, genetic and production cost

efficiencies to be exploited (Ingham *et al.*, 2007; Safari *et al.*, 2005). Consequently, many Australian farmers are shifting from traditional single-purpose to dual-purpose production systems (Pethick *et al.*, 2007). These producers are also supplementing lambs with protein-rich feeds to boost growth rates and returns from meat interests.

Dual-purpose production systems typically mate meat-type terminal sire breeds with a core flock of purebred Merino ewes (Greeff *et al.*, 2008; Rowe, 2010). This tiered crossbreeding approach utilises individual heterosis and improves lamb hardiness and growth (Gillespie and, Flanders, 2010; Thornton, 2010). Moreover, it combines desirable meat and wool production traits within all progeny (Ingham et al., 2007). Hence, paternal meat and growth characteristics are inherited by crossbred progeny along with maternal Merino wool characteristics. However, maternal genetic contributions have been suggested to be greater than paternal, being approximately 65% (Fogarty *et al.*, 2005b; Greeff et al., 2008). Paternal contributions also vary depending on sire breed selection.

**Wool**

Wool follicles (Figure 1.1) are epithelial derivatives of the epidermal tissue that are unique to sheep and the site of wool fibre proliferation (Galbraith, 2010). These extensively populate dermal surfaces and up to 0.1% of total lamb liveweight can be attributed to wool follicles (Liu and, Masters, 2000). Physiological differences allow wool follicles to be classified into three types (Hynd *et al.*, 1996; Rogers, 2006):

1. **Primary**, which mature within 60 days following conception and are arranged into trio patterns having one central and two lateral follicles. These characteristically synthesise coarser fibres.
2. **Secondary**, which develop 85 days after conception and are dependent on primary follicle locations for arrangement and size.
3. **Secondary-derived**, which evolve from secondary follicles after 105 days following conception to form ‘branch-like’ structures which share a dermal

opening. Both secondary and secondary-derived follicles characteristically synthesise finer fibres.

Wool fibres are proliferated from undifferentiated matrix cells located adjacent to the dermal papilla at a wool follicle's bulb (Galbraith, 2010; Purvis and, Franklin, 2005). This synthesis is limited by lysine and sulphur amino acid availability (Adams *et al.*, 2006; Plowman *et al.*, 2007). Lysine facilitates the required high DNA synthesis rate necessary during cell proliferation by maintaining histone levels (Plowman, 2007; Rogers and, Schlink, 2010). Sulphur amino acids are cysteine and methionine, with the latter potentially a precursor for cysteine resulting from transulphuration pathways (Rogers, 2006). Uptake of cysteine to the follicle requires active transportation, as with Na-dependent ASC transporter systems (Plowman, 2007). Cysteine functions in disulphide bonding and contributes to fibre structural rigidity and cellular differentiation within the follicle keratogenous zone (Galbraith, 2010; Plowman, 2007). Cellular differentiation provides the molecular components of a wool fibre's unique physical structure.

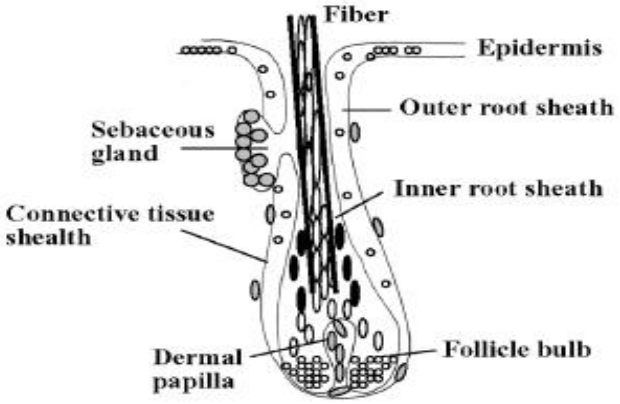


**Figure 1.1** A cross-sectional diagram of a wool follicle structure as per sheep dermal tissue (Thomas *et al.*, 2007).

The physical structures of wool fibres consist of an elongated spindle-shaped inner cortex layer covered with a thin scaled cuticle layer, as per Figure 1.2 (Adams *et al.*,

2000; Bringans *et al.*, 2007; Reis, 1992). Sometimes, in coarser fibres, a third 'Medulla' layer of air-filled cells within the cortex exists (Rogers and, Schlink, 2010). The cortex layer consists of longitudinally arrayed intermediate filaments embedded within and reinforcing an amorphous cellular matrix (Flanagan *et al.*, 2002; Reis, 1992; Rogers and, Schlink, 2010). This confers strength and rigidity characteristics on wool fibres (Koehn *et al.*, 2010). In contrast, the cuticle layer comprises of a several cell thick layers with a 'step down' pattern pointing towards the fibre tip (Woods and, Orwin, 1982). This results in fibre coefficient of friction to be lower on the base to tip direction (Sumner, 2005) and infers durability, abrasion resistance, shrinking and felting, and fabric handling characteristics (Koehn et al., 2010). The absence of longitudinal mechanical property contributions by cuticles promotes these characteristics (Reis, 1992).

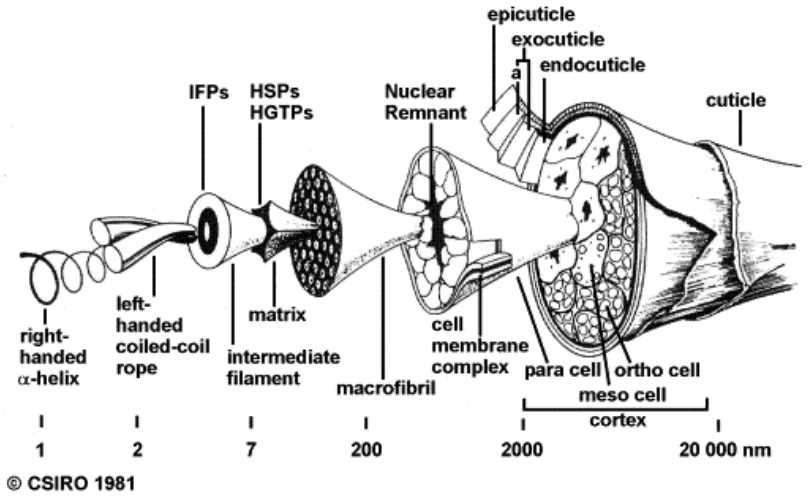


**Figure 1.2** Schematic diagram of a wool fibre with its major structural features labelled (Plowman, 2003)

Wool fibres can be classified as scleroproteins due to their inert fibrous protein composition and structural function (Liu and, Masters, 2003). Keratin proteins are predominant within wool fibre proteomes and contribute to approximately 90% of fibre weight (Purvis and, Franklin, 2005). These keratin proteins can be catagorised into two groups (Itenge *et al.*, 2009; Koehn et al., 2010; Plowman, 2007; Purvis and, Franklin, 2005):

1. **Keratin intermediate-filament proteins** (IFP), being structural alpha-helical microfibrils embedded within the matrix of KAP and contributing to 60% of total wool proteome. Although IFP are relatively homologous, they can be differentiated as acidic (Type I) or neutral-basic (Type II) IFPs.
2. **Keratin associated proteins** (KAP), contribute to the physical traits of wool fibres and can be differentiated by their primary amino acid constituents such as high glycine-tyrosine, high sulphur, or ultra-high sulphur KAPs.

The distribution and content of keratin proteins within wool fibres and physical structural layers contributes greatly to the physical traits, quality and commercial value of wool.

*Physical wool traits*

Wool quality and value are intrinsically linked by mutual dependence on market determined physical wool fibre parameters (Snowder *et al.*, 1997; Wood, 2003). Consequently, physical wool traits are routinely and objectively assessed to provide purchasers with an insight into wool quality, production performance and price discrimination. Producers also assess physical wool traits to quantify management choices that influence wool value. Although numerous physical traits can be tested, their contribution to ultimate quality and price varies (Mortimer *et al.*, 2010).

Mean fibre diameter (FD) is the average width of a wool fibre cross section measured as microns. This is the most important physical wool trait as it indicates the fineness to which yarn can be spun and its end applications (Rowe, 2010), and accordingly accounts for approximately 75% of the value of raw wool (Jones *et al.*, 2004; Mortimer et al., 2010). Fine FD is preferable to coarse wool and attracts a price premium. FD can be measured prior to processing using LASERSCAN equipment, although AIRFLOW and Optical Fibre Diameter Analysis (OFDA) approaches have previously been used (Botha and, Hunter, 2010; Tester, 2010).

Fibre diameter is not homogenous, but varies over an entire fleece (Wood, 2003). By monitoring this variation, wool quality can be assessed, with less variation desired and financially rewarded (Aylan-Parker and, McGregor, 2002; Snowder, 1992). Fibre diameter's standard deviation (SD) and coefficient of variation (CV) both present variation information. SD is derived from a normal distribution with higher SD values associated with greater variation. CV is a refinement of SD and allows wool with different FD to be compared (Baxter and, Cottle, 1997). Furthermore, 5% CV increments are equivalent to a one micron FD reduction during wool processing (Naylor *et al.*, 1995; Wood, 2010). Therefore, higher CV indicates lesser variation.

Spinning fineness (SF) adjusts FD with CV to provide indices of wool processing performance in terms of speed, cost and yarn evenness (Baxter and, Cottle, 1998; Naylor et al., 1995). Assessing SF can be advantageous as it compares wools in a standardised micron unit. And, low SF suggests increased wool quality (Holman, 2010; Malau-Aduli *et al.*, 2012a; Malau-Aduli and, Holman, 2010). However, scepticism overshadows the merits of SF, with general concerns that it overcomplicates FD and CV values which individually are easily interpretable.

Comfort factor (CF) refers to the percentage of wool fibres with diameters less than 30 microns, as those above 30 microns inherently irritate wearers (Naylor, 2010; Rogers and, Schlink, 2010). Therefore, high CF wools are premium and 95% CF a threshold of wearability (Malau-Aduli and, Deng Akuoch, 2010; Naylor et al., 1995). CF can be assessed with FD using LASERSCAN, however, in 2010, the CSIRO described the development of a 'Comfort Meter' which measures fabric surface anomalies using a sensory wire (Tester, 2010). While this provides accurate CF values, it can only do so after processing.

Fibre curvature (CURV) is objectively determined by LASERSCAN or OFDA technologies and describes crimp number, amplitude and aggression per unit of length as a single value (Hatcher and, Atkins, 2000b; Rogers, 2006). Low CURV wools are considered to be of greater quality as they perform better during scouring and carding by avoiding entanglements and breakages, respectively (Botha and, Hunter,

2010; Wang *et al.*, 2004). However, CURV can be altered using chemical wool treatments. For instance, CURV value increases have been observed with scouring compared to immersion in hot water (Botha and, Hunter, 2010). Nonetheless, CURV has been identified as a trait of increasing price discriminating importance as other physical wool traits are optimised (Hatcher and, Atkins, 2000b).

Clean fleece weight (YIELD) is the fibre content of raw wool excluding wax, suint, dust and vegetable matter contaminations (Jones et al., 2004; Rogers and, Schlink, 2010). Therefore YIELD refers to the usable wool percentage within an untreated raw product. YIELD underpins the monetary value of wool with consumers desiring higher YIELD (Jones et al., 2004). YIELD is generally measured using a representative 'core' sample to provide accurate interpretation of total fleece characteristics (Sidwell *et al.*, 1958).

*Wool proteome*

Proteomics is the process of protein identification within an organism or product's proteome (Riley *et al.*, 2010). It provides a holistic insight into cellular and physiological protein function and response to environment (Lippolis and, Reinhardt, 2008). Methods applied to proteomic analysis can be either gel or gel-free, although mass spectroscopy is the shared final stage used for qualification. This relies on comparisons between the large data output peaks and those identifiable by software such as MASCOT and Seaquest, which locates the closest matching known protein (Lippolis and, Reinhardt, 2008). However, at the moment, available databases are limited in their ovine and wool protein coverage (Clerens *et al.*, 2010).

Wool is predominantly comprised of proteins and therefore a prime candidate for proteomic analysis. Moreover, understanding wool fibre proteomes is thought to provide insights into physically measurable wool quality traits, discolouration, processing performance and response to management variables (Clerens et al., 2010; Poppi and, McLennan, 2010). It is hoped that this will improve production

management to better match wool quality to consumer demands. Accordingly, wool fibre proteome has been the focus of recent investigation:

- Clerens et al. (2010) used a combination of gel and gel-free approaches to identify 113 wool proteins, and showed that extraction techniques affect protein yields.
- Flanagan et al. (2002) found high sulphur protein patterns on a 2D gel detected variation between the wool fibre proteomes of Merino, Romney and Corriedale sheep breeds.
- Koehn et al. (2010) identified 108 proteins in purebred Merino wool cuticles alone, using a combination of chemical and enzymatic digestion methods. It was concluded that the cuticle is primarily keratin proteins and these contribute to cuticle structure and fibre characteristics, although some cortex protein contamination was assumed.
- Plowman et al. (2010) showed IFP to dominate 2D gels and impede the identification of less abundant wool proteins.
- Plowman et al. (2000) associated several high sulphur proteins with CURV using 2D gel methods to highlight the role of wool proteome with physical wool traits.

The main limitation to proteomic research with wool is the relative infancy of this method, as reflected within web databases (Plowman, 2003, 2007) and the lack of a standardised wool proteomics protocol (Schulze and, Usadel, 2010). However, proteomics is undergoing constant development with instruments improving, costs reducing, and database coverage expanding (Bendixen *et al.*, 2011). Consequently, proteomics is thought to have an increasing role in improving livestock production efficiencies not just in wool but across the board (Thornton, 2010).

**Meat**

Lamb carcasses consist of four primary tissue types:

1. **Skeletal tissue**, which increases with lamb maturation and provides structural integrity (Campbell *et al.*, 2002).
2. **Organ tissue**, which is consumed less so in Western cultures and is essentially a by-product of meat production (Park and, Washington, 1993).
3. **Adipose tissue**, primarily an energy storage tissue being comprised primarily of energy dense lipids and its consumption is often avoidable by trimming subcutaneous fat but unavoidable as intramuscular fat (Geesink and, Zerby, 2010).
4. **Muscle tissue**, which is the main consumable and high valued lamb carcass component with its quality and yield a key production focus. Therefore, this will be discussed further in this review unlike the previous tissue types listed.

Muscle tissue subunits are myofibrils which consist predominantly of protein, albeit glycogen and lipid stores which are present in lesser amounts. Myofibrils are arranged into bundles or sheets which are surrounded by a perimysium (Campbell et al., 2002; Warner *et al.*, 2007). An entire muscle is insheathed by an epimysium which contributes dense connective tissues constituting tendons (Geesink and, Zerby, 2010). These are collectively termed 'connective tissue' and are a network of collagen and elastin fibres which permit muscle growth and transmittance of contractive forces (Allingham *et al.*, 2006; Wallace, 1979).

Depending on their location within a lamb carcass, myofibrils vary between three types (Geesink and, Zerby, 2010; Reggiani and, Mascarello, 2004; Warner et al., 2007):

1. **Type 1 / Slow Twitch**, using aerobic metabolism and resistant to fatigue. These are found in higher levels in postural muscles.

2. **Type 2A / Fast Twitch**, similar to Type 1. These use aerobic metabolism, are resistant to fatigue and found mostly in limb muscles.
3. **Type 2B / Fast Twitch**, using anaerobic or glycolytic metabolism are rapidly fatigued and found in locomotive muscles.

Myofibril type affects lamb meat sensory quality (Hopkins *et al.*, 2007c; Warner *et al.*, 2010). However, it is the comparatively minor lipid content of muscle tissue which acts as the primary determinant of meat quality. Similarly, adipose and organ tissue lipid profile underpins their respective qualities.

Lipids are composed of fatty acids (FA) which can be found as free FA or as triacylglycerols wherein three FA are attached to a glycerol molecule (Campbell and, Farrell, 2012; Gurr and, James, 1972). It is this reversible transformation of triacylglycerols to free FA from which lipid energy storage capacity is based. This process is allowed by individual FA structure being amphiphilic; having a hydrophilic carboxyl group attached to a hydrophobic carbon chain (Webb and, O'Neill, 2008). The length and bond types present in these carbon chains is used to differentiate FA nomenclature and function (Webb and, O'Neill, 2008). For instance, long chain FAs have carbon chains ranging from 13 to 21 carbon atoms, whereas short chain FAs have less than 13. Furthermore, saturated FAs (SFA) have carbon chains with no double bonds unlike unsaturated FAs (UFA), which can be either monounsaturated (MUFA) or polyunsaturated (PUFA) if only one or several double bonds exist respectively (Gurr and, James, 1972).

FAs are distributed throughout ruminant tissues with muscle FA deposits having major implications on meat quality. However, FA profile differences have been found between tissue deposit sites in ruminants. For instance, muscle FA profiles are largely long chain FA (Aurousseau *et al.*, 2007a), whereas adipose tissue has comparatively greater SFA content (Wood *et al.*, 2008). However, Bas and Morand-Fehr (2000) conducted a meta-analysis demonstrating that overall adipose and muscle FA profiles are relatively similar having only minor differences (Wood et al., 2008). These differences stem from the preferential incorporation of essential FA

into muscle compared to adipose tissues. Wood et al. (2008) reported that linoleic acid is principally in muscle tissue rather than adipose. Differences between organ FA profiles remain relatively unexplored in lambs and other key ruminant species.

*Sensory meat quality traits: tenderness, juiciness, flavour, and appearance*

Lamb quality quantified by consumer's tangible senses or perceived product characteristics is referred to as 'sensory quality'. These involve several aspects of stimulation:

Tenderness is the main determinant of meat sensory quality having price discrimination favouring tender meat cuts (Pethick et al., 2010a; Warner et al., 2010). Tenderness is affected by the amount and solubility of myofibril connective tissues as these affect residual adhesion during cooking (Allingham et al., 2006; Warner et al., 2010). Intramuscular fat percentage (IMF) also affects tenderness, with 3% IMF shown as the threshold of acceptable tenderness for Australian consumers (Hopkins et al., 2007c).

Juiciness refers to the sensation arising from fluid release and salivation stimulation upon meat cut mastication (Warner et al., 2007). Consequently, juiciness is a function of tissue water holding capacity which is related to IMF, as IMF retains fluid even after cooking. Similarly with tenderness, juiciness IMF threshold is 3% (Hopkins et al., 2007c).

Flavour is the combination of taste and smell sense stimulations arising upon consumption (Geesink and, Zerby, 2010). Wallace et al. (1979) describes flavour as underpinning Australian consumer satisfaction. Tissue FA profile directly affects flavour, with methyloctanoic and 4-methylnonoic acids which contribute to lambs' distinctive flavour (Watkins *et al.*). IMF also affects flavour, with 5% considered ideal (Hopkins et al., 2007c). However, lamb flavour acceptability and satisfaction ultimately depends on the consumer's cultural and culinary backgrounds.

Appearance is intrinsically linked with shelf-life and saleability, being consumer's first encounter with a lamb product (Hopkins et al., 2007c; Refshauge *et al.*, 2008). Muscle tissue appearance is preferably bright red, arising from myoglobin oxygenation and tissue freshness as metmyoglobin forms as meat ages and alters colour towards greyish-brown (Geesink and, Zerby, 2010). Adipose tissue colour contributes to appearance and is ideally white. However, adipose whiteness is positively associated with SFA content, becoming yellow with increased UFA and beta-carotene contents. Similarly, shelf-life is promoted by SFA content which is relatively resistant to oxidation (Webb and, O'Neill, 2008). SFA and adipose tissue firmness are positively associated due to UFA and SFA melting point differences. These differences allow melting point to be used to objectively assess FA composition (Malau-Aduli *et al.*, 2000c; Perry *et al.*, 1998).

Sensory quality can be directly analysed using tasting panels, which rely on large, expensive and time-consuming protocols to achieve statistical significance (Geesink and, Zerby, 2010; Pethick et al., 2010a). Therefore, indirect measurements which have strong associations, such as tissue FA composition, content and IMF, are widely utilised instead.

*Nutritional meat qualities*

Lamb nutritional quality revolves around increased human health benefits and reduction of antagonists associated with consumption. This stems from lamb meat iron and zinc (Pannier *et al.*, 2010), and vitamin E (Ponnampalam *et al.*, 2012) content and ability to provide nutritional sustenance. However, FA content and composition significantly contribute to its actual and perceived nutritional quality.

SFA has been widely acknowledged as a causal factor of cardiovascular disease, particularly in developed countries (Enser *et al.*, 1998; Pannier et al., 2010). SFA has also been identified as an antagonistic agent of several types of cancer. Lamb is considered a rich source of SFA. Consequently, producers are actively seeking ways of reducing SFA content of meat, in favour of leaner meat products. However, this

focus has counterintuitive affects on the UFA content, especially PUFA levels. Several PUFA are considered beneficial to human health (Raes *et al.*, 2004):

- Eicosapentaenoic acid (20:5ω3; EPA).
- Docosapentaenoic acid (22:5ω3; DPA).
- Arachidonic acid (20:4ω3; ARA).
- Docosahexaenoic acid (22:6ω3; DHA).

Therefore, many producers and researchers have prudently adopted SFA/PUFA ratios as indices of nutritional quality and health.

Interest in Omega-3 (n-3) PUFAs has increased over recent decades. This arises from n-3 having a strong association with improved human health upon consumption, by reducing coronary heart disease risk and promoting mental and infant development (Howe *et al.*, 2006). Hence, as a group, health professionals are advocating increased n-3 intakes within typical diets. Dietary n-3 content is commonly reported as a ratio with Omega-6 (n-6) PUFA as n-6 competes with n-3 for rate-limiting enzymes and doing so counters n-3 action. Nonetheless, a healthy human diet should contain both n-6 and n-3, ideally at a 4:1 ratio (Howe et al., 2006). As n-6 is readily obtained with typical diets, it is maintaining n-3 levels which present a greater challenge. Consumption of lamb can assist in this goal, even with lamb being a poorer source of n-3 compared to oily fish, as lamb is widely and regularly consumed (Scollan *et al.*, 2001).

Linoleic acid has biological roles in both structural and synthetic capacities, and its consumption has been associated with reduced serum cholesterol and resultant improvements in cardiovascular health, diabetes and cancer risks, and immune function (O Quinn *et al.*, 2000). Ruminant meat is considered a linoleic acid-rich source, albeit as conjugated linoleic acid (CLA). This derives from CLA being sourced from dietary FA and is synthesised *de novo* in the adipose tissue.

**Productivity**

The ability to objectively assess lamb response to management strategies is vital to streamlining selective breeding programs and supplementary diets (Afolayan *et al.*, 2006) and maximising productivity. Productivity also is derived from heightened animal health and welfare (Hogan and, Phillips, 2008), making it a key parameter in quantification along with sociological considerations (Peterson *et al.*, 2008).

*Body conformation traits*

Understanding lamb body conformation, growth and liveweights provide objective means for producers to streamline selective breeding (Mortimer *et al.*, 2009) and lamb productivity (López-Carlos *et al.*, 2010).

Liveweight is a rapid and precise measurement which provides approximations of lamb productivity, market value and growth response, generally calculated as average daily gain (ADG). Liveweight can be assessed using electronic sheep scales or weighbridges (Cam *et al.*, 2010), yet in situations where these are unavailable, measurement of body conformation traits can provide comparable values (Afolayan et al., 2006; López-Carlos et al., 2010; Sowande and, Sobola, 2008). Furthermore, body conformation traits avail lamb growth quantification in regions of economic interest, such as the valuable meat cuts. Widely monitored body conformation traits include:

1) **Chest girth**, being a lamb's circumference just behind its forelegs (Cam et al., 2010).
2) **Withers height**, the gap between the ground and the highest peak over the scapulae (Sowande and, Sobola, 2008).
3) **Body length**, the span between the scapulae and the pubic bone (Sowande and, Sobola, 2008) .

Locating these reference points is vital for maintaining information integrity. Measuring while lambs are restrained in an unforced position with head erect and all four legs firmly planted is also important for ensuring accuracy (López-Carlos et al., 2010).

Body condition score (BCS) is a subjective evaluation of subcutaneous adipose cover or fatness which indicates lamb productivity and health. This relies on expertise to make consistent judgements when palpating lambs' ribs fat covering (McLeod et al., 2010; Phythian *et al.*, 2011). Using a single operator for all comparable measurements and avoiding wool interference can reduce Type I errors. BCS can then be quantified on an incremental 1-5 point scale (Hogan and, Phillips, 2008; Jefferies, 1961):

1. Individual ribs can be readily felt and no tissue coverage.
2. Individual ribs felt yet some tissue present.
3. Ribs felt through significant tissue coverage.
4. Ribs just felt and covering tissue shows fluidity.
5. Ribs cannot be felt through very fluid tissue coverage.

*Haematological metabolites*

Lamb health, welfare and productivity are three fundamental concerns to producers and animal scientists. Each of these concerns can be objectively assessed by analysing haematological metabolite concentrations (Antunovic *et al.*, 2002; Ford, 1974; Hegarty *et al.*, 2006a). These haematological metabolites permit actual lamb response to experimental treatments and management practises to be observed (Ololade and, Mowat, 1975; Trenkle, 1978).

Haematological metabolites routinely assayed include:

- **Creatine kinase** – marker of muscle damage (Russell and, Roussel, 2007) and myocardial infarctions (McLeish and, Kenyon, 2005).

- **Aspartate aminotransferase** – marker of liver disease (Center, 2007) and fasciolosis in sheep (Ferre *et al.*, 1995).
- **Glutamate dehydrogenase** – marker of liver damage (Braun *et al.*, 2010), especially in sheep following hepatic intoxication with various agents (Ford, 1974).
- **Gamma-glutamyl transferase** – marker of cholestasis (Whitfield, 2001) and liver disease in sheep (Braun *et al.*, 1983).
- **Bilirubin** – marker of blood and liver disease, such as facial eczema in sheep (Braun et al., 2010; Russell and, Roussel, 2007).
- **Creatinine** – marker of lamb kidney health (Braun et al., 2010) and total muscle mass (Hegarty *et al.*, 2006b).
- **Total protein -** marker of ingested protein; Albumin, marker of liver, kidney and blood disease and long term dietary trends (Meijers *et al.*, 2008; Russell, 1982; Sargison and, Scott, 2010); and, Globulin marker of liver disease or chronic antigenic stimulation in sheep (Russell and, Roussel, 2007).
- **Beta-hydroxybutyrate** – marker of nutrition/stress levels (Laeger *et al.*, 2010).
- **Urea** – marker of short term dietary nitrogen supply (Hegarty et al., 2006b; Huntington and, Archibeque, 2000) and liver disease, albeit poor (Braun et al., 2010).
- **Glucose** – marker of glucose use rate and supply (Annison and, White, 1961) and lamb health, although only at time of sampling (Filipovic *et al.*, 2011).
- **Non-esterified fatty acids** – marker of liver function and nutritional status (Annison, 1960).
- **Cortisol** – marker of stress and responsiveness to nutritional changes (Bradshaw *et al.*, 1996; Cook, 1997).

Haematological minerals and electrolytes contribute to normal lamb function as they are vital structural material that optimise biochemical reactions and maintain cellular and osmotic integrity (Campbell et al., 2002; Russell, 1982). Therefore, monitoring their concentrations provides valuable insight into lamb health and productivity (Antunovic et al., 2002). Minerals and electrolytes that are of particular interests

include calcium, magnesium, phosphate, sodium, potassium and chloride (Campbell et al., 2002; Russell and, Roussel, 2007).

**Genetics**

Genetics is the study of DNA and genes, which are the underlying inherited blueprint determinants of lamb productivity and product quality potentials. While many techniques presently exist which can manipulate and manage lamb genomes, most of them are impractical for large scale lamb operations or give rise to social concerns and fear of the unknown associated with genetic engineering and genetically modified products. Consequently, this chapter discusses only sire breed and its interaction with sex, both of which are routinely managed by Australian producers to achieve production and product quality goals.

*Sex*

Male and female genetic differences arise from sex chromosome variations – females having XX and males XY (Kodric-Brown and, Brown, 1984; Preston *et al.*, 2001). This definite variation affects lamb productivity through interrelated endocrinological, physiological and development divergences.

Endocrinological differences between sexes are epitomised by testosterone and oestrogen levels, being prolific male and female hormones respectively (Slen and, Connell, 1958). This variation has been identified as the root cause of large variation in lamb productivity:

- Slen and Connel (1958) found testosterone to have a strong positive association with wool yield and fibre coarseness, and oestrogen with suppressing wool growth and improved physical quality traits. Other studies have reflected this finding (Corbett, 1979; Egan and, Russell, 1981; Malau-Aduli and, Deng Akuoch, 2010; Malau-Aduli and, Holman, 2010; Wallace, 1979).

- Corbett et al. (1979) describes testosterone as a growth and liveweight promotant. Increases are primarily expressed in the bulking of muscles in proximity to head and neck regions (Sowande and, Sobola, 2008; Warner et al., 2007). This has been supported by Afolayan et al. (2006) who reported that testosterone levels in wethers were artificially increased.
- Cake et al. (2007) has associated oestrogen level with bone growth plate closure and the ewe's smaller mature anatomical proportions compared to the wether's and ram's. This causes differences in structural support available to support large liveweights and growth rates (Ponnampalam *et al.*, 2007; Sowande and, Sobola, 2008; Warner et al., 2007).

Care must be taken when using wethers as representative males in any lamb trial. This is due to wether's being castrated male sheep and hence have compromised endocrine systems which produce less testosterone than intact males (Egan and, Russell, 1981; Jenkins *et al.*, 1988). However, wethers are common in Australian flocks and therefore included in many trials to increase result practicality and representation. Similarly, trials must note that when ewes are included, their progression through reproduction, pregnancy and lactation states affects nutrient partitioning and requirements, and physiology (Reis, 1992; Rogers and, Schlink, 2010). Consequently, research comparing males and females as a rule, use dry or non-lactating ewes.

Most of the observed effects of sex on lamb productivity are due to physiological differences in size and liveweight. This is reinforced by similarity in feed intakes per body mass (Rogers and, Schlink, 2010) and mature rumen function (De Deposito *et al.*, 2009) between sexes.

Sex has been suggested to affect lamb meat quality. Pethick et al. (2010b) showed that female lambs had 0.20% higher IMF compared to males and has been supported by other studies suggesting comparative 'fattiness' in ewes (Mezoszentgyorgyi *et al.*, 2001). However, Horcada et al. (1998) and De Deposito et al. (2009) both suggest

that sex had no effect on lipid composition. This divergence may have arisen from differences in protocol and lamb breeds or sire breeds used.

*Breed*

Today's diverse sheep breeds were developed from intensive natural selection and domestication (Leymaster, 2001). Consequently, innumerable adaptations and characteristics have developed which are unique to specific sheep breeds. These genetically based traits can be exploited by farmers to match flock characteristics with productivity and product quality goals (McGuirk *et al.*, 1978; Warner et al., 2007). This can be achieved by selectively breeding or systematic crossbreeding, which is more common in dual-purpose operations as crossbred lambs 'as a rule' outperform their purebred parental breeds (Santos-Silva *et al.*, 2002b). However, the extent of this effect depends on breed genetic potential and appropriate sire breed selection.

The introduction of paternal genetic traits into progeny is not straight-forward. Contribution and progeny expression differs between individual rams and breeds. Therefore, genetic markers and quantitative trait loci have been widely used to predict genetic potential and develop estimated breeding values (EBV) and breeding indices (Bidinost *et al.*, 2008; Fogarty *et al.*, 2006). This aids in simplifying crossbreeding, especially when several traits contribute to productivity or producers have dual-purpose product focus (Brash *et al.*, 1994; Ingham et al., 2007). LAMBPLAN and MERINOSELECT are two such EBV databases used extensively by Australian producers (Hopkins et al., 2007b).

To recap, Australian dual-purpose lamb producers routinely crossbreed meat-type terminal sires with a core purebred Merino flock to optimise productivity. Consequently, numerous studies have been undertaken on the effect of sire breed on lamb productivity and product quality, for example:

- Fogarty et al. (2005a) found sire breed to affect lamb post-weaning liveweight, hot carcass weights, carcass fat levels, eye muscle area and physical wool quality traits CFW and FD.

- Hopkins et al. (2007a) found lamb sire breed to affect wool production, with purebred Merino lambs producing 0.4 kg and 1.5 kg more wool than their Border Leicester- and Poll Dorset-sired counterparts respectively, and purebred Merinos were the lightest compared to crossbred lambs.
- Ingham et al. (2007) estimated the heritability of lamb growth traits (0.07-0.29), meat colour and pH (0.10-0.23), carcass fat and muscle traits (0.32-0.47), and wool traits (0.36-0.55) using progeny data from purebred Merino ewes crossed with Border Leicester, East Friesian, Finn sheep, Coopworth, White Suffolk, Corriedale and Borroloola Leicester rams.
- Malau-Aduli et al. (2012b) showed sire breed affected wool quality, with spinning fineness lowest in Texel-sired lambs, followed by Suffolk- and Dorset and Merino-sired lambs sequentially.
- Pethick et al. (2010b) found purebred Merinos had 0.42% higher IMF compared to terminal sired lambs
- Ponnampalam et al. (2007) describes carcass fatness traits in lambs with Border Leicester genetics being more fatty than lambs with Merino or Poll Dorset genetics.
- Scales et al. (2000) showed Poll Dorset-sired lambs outgrew purebred Merinos until the trial concluded when lambs were 12 months old. Texel- and Poll Dorset-sired lambs had larger eye muscle size compared to other sire breeds (Merino, Border Leicester, Oxford Down, and Suffolk).

From these, we can conclude that the use of different sire breeds in crossbreeding programs affects progeny genomes and subsequently phenotypes and internal nutrient pathways. Crossbreeding purebred Merinos with meat type sire breeds improves liveweight and growth traits while retarding wool production and quality. Sire breed response to management strategies, such as supplementation, varies.

The genetic diversity between each homozygous parental breed combined in crossbred progeny increases its relative heterozygosity resulting in an effect known as heterosis (Leymaster, 2001). Subsequently, heterosis causes first-generation ($F_1$)

lambs to outperform their sires and dams (McLeod et al., 2010; Pitchford, 1992). Petrovic et al. (2011) found heterosis effects were highest with genetic traits with low heritability. However, contention exists regarding the source of heterosis as confirmation remains elusive.

**Nutrition**

Lamb genetic and productive potential can only be reached with adequate nutrition (Pethick et al., 2007) and when basal diets are unable to match requirements the use of supplementation is typical. Australian dual-purpose lamb producer's supplements are generally protein- and/or energy-rich to promote ideal productivity and product quality (Hogan and, Phillips, 2008). However, supplementation can exponentially increase production costs. Therefore, understanding lamb rumen function and best supplementation levels is imperative.

*Rumen metabolism*

Carbohydrates are the principal source of energy to ruminants and necessary to support lamb function and productivity. This originates from carbohydrates composition as simple or complex sugars. Simple sugars can be readily metabolised by mammals unlike complex sugars which require more intensive metabolism, such as cellulose and lignin (Nafikov and, Beitz, 2007). Ruminants are best positioned to metabolise complex carbohydrates as their rumen contains microorganisms which ferment these sugars (Laeger et al., 2010). By-products of this fermentation include volatile FAs, mostly acetic acid (Laeger et al., 2010), and simple sugars available for absorption, although only 5-20% of that originally ingested (Armstrong and, Smithard, 1979; Nafikov and, Beitz, 2007). Consequently, rumen microbes and ruminants share a symbiotic relationship with complex carbohydrate metabolism dependent on ruminal digestion.

Lamb tissue protein metabolism is similar to other mammals, requiring 10 essential amino acids (AA) which must be exogenously sourced (Armstrong and, Smithard, 1979). Rumen interactions with dietary protein provide two such exogenous sources:

1. **Undegradable protein**, being dietary protein which escapes deamination within the rumen and reaches the duodenum for absorption relatively intact (Orskov, 1982). This passage depends on protein rate of passage, solubility in water, heat treatment, and nitrogen, sulphur and energy availability (AAC, 1990). Its usefulness also varies with the extent to which it can be absorbed within the duodenum and its AA profile.
2. **Microbial protein**, being dietary protein soluble fractions and contributing nitrogenous compounds, primarily as ammonia, to the raw precursors required for rumen microbes to synthesis AA and meet their structural requirements (Corbett and, Ball, 2002; Dove, 2010). As these microbes are digested with remaining digesta, they release AA from absorption within the duodenum. This process allows ruminants to convert elemental nitrogen into AA. Therefore, protein can be sourced not only from dietary protein but non-protein nitrogen arriving through dietary supplementation or nitrogen recycling, as urea. Consequently, dietary AA profile does not reflect that actually absorbed (Lapierre and, Lobley, 2001; Orskov, 1982).

Lambs, being ruminants, have the capacity to recover waste nitrogen as microbial protein. This is based on liver excretion of haematological urea derived from ammonia (Bligh and, Dyer, 1959) which is then transported to the rumen as opposed to being removed by the kidneys (Lapierre and, Lobley, 2001). Haematological urea enters the rumen with saliva or through the rumen wall (Boďa *et al.*, 1986). However, entry is dependent on rumen AA and blood urea content, as urea absorption decreased as protein intake increases from endocrinological marker interaction (Marini *et al.*, 2004).

Unlike monogastrics, FA ingested vastly differs from those absorbed in ruminants (Bas and, Morand-Fehr, 2000; Doreau *et al.*, 2011). This is thought to be an

adaptation to conserve FA, as natural ruminant diets are relatively low in lipid. This difference is limited somewhat to FA profile with Lock et al. (2006) and Jenkins (1993) stating that ingested and absorbed FA levels are relatively comparable. However, a higher trend of absorption to ingestion in ruminants has been observed, except with lipid-rich diets (Doreau and, Ferlay, 1994). The basis of these characteristics is rumen function affecting lipid metabolism.

Lipid metabolism in the rumen is sequential. The initial phase shortly follows consumption, during which the majority of lipids are lipolysed or 'broken down' into free FA (Doreau et al., 2011). This is chiefly through rumen microbial action, particularly *Anaerovibrio lipolytica* and *Butyrivibrio fibrosolvens* and several other identified microbial strains which produce lipase and esterase (Jenkins, 1993; Lourenco *et al.*, 2010). Fodder and consumed plant material also makes minor lipase contributions to assist in lipolysis (Lourenco et al., 2010). Along with free FA, other products of lipolysis include glycerol and glactose which are both fermented to propionic and butyric acid end-products. After lipolysis, FA undergoes biohydrogenation and isomerisation.

Biohydrogenation and isomerisation have been the topics of review (Jenkins, 1993). As an overview, biohydrogenation and isomerisation are the processes in which free FA are hydrogenated to SFA through ruminal microbial and protozoa activity, the latter's role arises through predation of microbes rather than a direct involvement (Doreau et al., 2011). The first step of biohydrogenation depends on the isomerisation reactions of isomerase. Isomerase is only functional when a FA has a free carboxyl group, or a PUFA has a cis-12 diene double bond configuration (Jenkins, 1993). It acts to shift FA double bond, in free UFA being the cis-12 double bond so the UFA becomes a trans-11 isomer. This isomer can then be hydrogenated, or saturated, through microbial reductase activity. The theoretical end-product of biohydrogenation and isomerisation is stearic acid and other SFA. Instead, numerous MUFA isomers also pass to the duodenum for absorption (Jenkins, 1993). For instance, CLA isomers, such as health promoting c9t11CLA and t10c12CLA (O Quinn

et al., 2000), are formed through incomplete hydrogenation of linoleic acid, itself a primary substrate for biohydrogenation (Lourenco et al., 2010).

*Feed intake*

Feed acquisition is a significant expense to Australian dual-purpose lamb producers (Fogarty *et al.*, 2009; van der Werf, 2004). Moreover, this cost is thought to be compounded with future:

- **Climatic change**, with increased drought periods predicted in key production regions affecting feed production and availability (Nardone *et al.*, 2010).
- **Competition**, with alternative feed uses, such as with biofuel production or other agricultural sectors (Hegarty, 2012).
- **Changing land usage**, caused by urban sprawl or environmental protection limiting production expansion potential (Godfray *et al.*, 2010).

It is in producers best interest to reduce lamb feed intake requirements to achieve similar productivity. Lamb sex and sire breed have been found to have no effect on feed intake once adjusted for liveweight as feed intake and liveweight share a positive association (Hill, 2012). In contrast, feed supplementation has been found to affect feed intake levels. Dolye et al. (1988) found that the voluntary intake of oaten hay by weaner lambs decreased with increased oat grain and sunflower meal supplementation. Ferrell et al. (1999) on the other hand, found that feed intake increased with protein-rich supplementation. Differences in response have been due to the fact that supplementation increases when intake only when basal diets are NDF-poor and *vice versa* (Moore *et al.*, 1999a; Salisbury *et al.*, 2004).

Greater understanding of lamb feed use efficiency and requirements would aid dual-purpose producers to maximise productivity and profits. At present, several models exist which calculate residual feed intake, feed conversion ratios and specific growth rates (Knott *et al.*, 2008; Lewis and, Emmans, 2010). These models provide values adjusted by liveweight, productivity, feed and supplementation parameters to assist accurate interpretation of feed usage (Knott et al., 2008).

*Supplementation*

Supplementation ensures strategic levels of protein, lipids and carbohydrates that are required to achieve optimal productivity and product quality are met (Hogan and, Phillips, 2008). The extent to which supplementation affects productivity and product quality is a function of supplement type, level and its interactions with the basal diet. Previous studies in published literature have investigated these factors with several traditional supplements and assessing various parameters of productivity and product quality.

Many supplements used focus primarily on improving growth and liveweight productivity:

- Karlsson et al. (2011) found supplementing lamb barley-based diets with peas and rapeseed cakes improved growth rates unlike including hempseed cake.
- Malau-Aduli et al. (2009b) demonstrated that canola supplemented lambs grew higher liveweights than their lupin-fed counterparts by 40 kg to 37 kg respectively.
- Moore et al. (1999b) conducted a meta-analysis of 66 publications and found that supplementation decreased feed intake when supplement total digestible nutrition was less than 0.7% cattle liveweight. Furthermore, supplementation did not always increase growth, but when it did, it was not related to the supplement's total digestible nutrients.
- Ponnampalam et al. (2002) showed that lambs supplemented with lupin or fish meal had higher slaughter weights and hot carcass weights than those fed barley.

Meat and wool quality traits are generally affected by supplementation either intentionally or as a side-effect:

- Demirel et al. (2004) found that the nutritional quality of lamb meat could be improved by increasing their intake of PUFA, using fish oil and whole linseed based supplements.
- Kitessa et al. (2010) described how equivalent n-3 PUFA content within lamb muscle tissue could be achieved in pasture finished lambs by supplementing indoor-finished lambs with linseed.
- Malau-Aduli et al. (2012; 2009b; 2009e) and Malau-Aduli and Deng Akuoch (2012) showed that physical wool quality traits were not affected by supplementing lambs with canola or lupin meals.
- Masters et al. (1999) showed that weaners fed canola meal produced 11% more wool than those fed lupins, due to induced higher rates of protein synthesis in the skin.
- Nute et al. (2007) found that lambs supplemented with linseed oil had the highest 18:3ω3 content in muscle tissue, rating for lamb flavour, and overall liking compared to those supplemented with fish oil or marine algae. These latter supplements were noted as producing 'putty' and 'fishy' odours.
- Ponnampalam et al. (2002) showed that long chain n-3 PUFA content in lamb muscle tissue were highest with fish meal supplementation, whereas n-6 PUFA were highest with lupin supplements.

Identification of optimal supplementation levels that impact on productivity and quality provides monetary incentives, however much depends on supplement type and basal diet:

- French et al. (2000) describes an increase in total FA, SFA and n-6:n-3 ratio, and a decrease in CLA and PUFA:SFA ratio with increasing concentrate proportions within continental crossbred steer diets.

- Fisher et al. (2000) found Suffolk crossbred lambs fed concentrates had higher 18:3ω6 and 20:4ω6 content compared with forage fed lambs which had greater 18:3ω3 and 20:5ω3 contents.
- Hentz et al. (2012) showed that basal forage DM intake in Polwarth x Texel wethers decreased progressively with increased canola meal supplementation levels.
- Rowe et al. (1989) found that in purebred grazing Merinos, fibre diameter (18.6-21.1 µm) and staple strength increased as grain supplementation levels increased from 0 to 750 g/day.
- Ryan et al. (2007) supplemented Boer crossbred goats with concentrates and reported increases in liveweight, hot carcass weight, dressing percentage, rib-eye area, and carcass length with increased levels of concentrate supplementation compared with pasture-fed equivalents. Also, carcasses of goats on low concentrate supplementation level had higher percentage of trimmed shoulder than their counterparts on medium supplementation levels.

These studies highlight the immensity of choice facing Australian dual-purpose lamb producers wishing to use supplementation to manage productivity and product quality.

***Spirulina***

*Spirulina* (*Arthrospira sp.*) is an edible filamentous, spiral shaped cyanobacterium (Becker, 2007; Gouveia *et al.*, 2008). It is naturally found in the alkaline lakes of Mexico and Africa (Shimamatsu, 2004) where it has a long history as an ancient food source. *Spirulina* was rediscovered relatively recently by Leonard and Compere in the 1960s (Shimamatsu, 2004) and since then, has become commercially mass produced world-wide (Muhling *et al.*, 2005).

*Spirulina* is grown within nutrient-rich liquid medium (Chaiklahan *et al.*, 2010; Hasdai and, Ben Ghedalia, 1981); hence, it can be produced with high land-use efficiency. For instance, *Spirulina* out yields many traditional livestock feeds, including wheat, corn, barley and soybeans, in protein output per land unit (Dismukes *et al.*, 2008; Kulpys *et al.*, 2009). Furthermore, *Spirulina* can be grown using desalinator wastewater (Volkmann *et al.*, 2008) or animal faecal waste enriched growth medium. This latter medium has been trialled using pig (Chaiklahan et al., 2010) and cattle (Mitchell and, Richmond, 1988) faecal waste, and resultant *Spirulina* was found safe to consume. These highlight *Spirulina's* aptness to cost effectively treat waste and rescue nutrients (Saxena *et al.*, 1983).

*Spirulina* is relatively expensive to produce and purchase compared with other livestock feeds. This undermines its practical use for many animal production systems. *Spirulina's* palatability, dry powdery form, and smell further limit its practicality (Becker, 2007). However, ongoing developments in low-cost growth medium and improved operational management of *Spirulina's* nutrient use efficiency and growth rates are thought to reduce its cost (Peiretti and, Meineri, 2011; Raoof *et al.*, 2006; Shimamatsu, 2004). Likewise, research into *Spirulina* delivery methods and impact on product quality allows greater understanding of its practicality.

*Spirulina* is nutrient-rich. It contains all essential amino acids, vitamins and minerals. *Spirulina* is also a rich source of carotenoids and fatty acids, especially gamma-linolenic acid which has been associated with several health benefits (Howe et al., 2006). *Spirulina's* high protein content distinguishes it as a livestock feed or supplement (Belay *et al.*, 1993; Doreau *et al.*, 2010). The nutritional value of *Spirulina* has been the topic of several reviews (Belay et al., 1993; Ciferri, 1983; Diraman *et al.*, 2009). Similarly, variation in *Spirulina's* nutritional values between production systems has also been extensively reviewed (Babadzhanov *et al.*, 2004; Mata *et al.*, 2010; Tokusoglu and, Unal, 2003; Vonshak and, Richmond, 1988).

*Chickens*

The effect *Spirulina* has on chicken growth depends on the feed it replaces in a ration. For instance, Ross and Dominy (1990) observed declining growth rates when *Spirulina* replaced dehulled soybean meal. In contrast, Saxena et al. (1983) and Venkataraman et al. (1994) found *Spirulina* to have no affect on growth when replacing groundnut cake and fishmeal, respectively. Toyomizu et al. (2001) found *Spirulina* levels of 50-100 g/kg of feed ration maintained typical growth rate, but at levels exceeding 200 g/kg would reduce growth.

*Spirulina* has been associated with improved chicken health and cost effective production. For instance, chickens consuming *Spirulina* were found to have increased macrophage and overall mononuclear phagocyte system functionality, both indicating enhanced disease resistance (Al-Batshan *et al.*, 2001; Qureshi *et al.*, 1996). Qureshi et al. (1996) found chicken health to be improved with just low *Spirulina* levels, even at 10 g/kg feed ration. Venkataraman et al. (1994) showed typical vitamin-mineral premixes used in chicken feed rations can be omitted when *Spirulina* was included.

Chicken product quality can be tailored towards consumer preferences using *Spirulina*. Total cholesterol content of eggs, for example, can be lowered with *Spirulina* provision due to its high antioxidant and n-3 PUFA content (Rajesha *et al.*, 2011; Sujatha and, Narahari, 2011). Yolk colour can also be incrementally intensified with increased *Spirulina* levels (Ross and, Dominy, 1990; Sujatha and, Narahari, 2011). This results from *Spirulina's* high content of zeaxanthin, xanthophylls and other carotenoids pigments, particularly beta-carotene, all of which accumulate within yolks (Anderson *et al.*, 1991; Takashi, 2003). These same compounds accumulate within chicken muscle tissue. Hence, both Toyomizu et al. (2001) and Venkataraman et al. (1994) found increased muscle yellowness and redness with proportional increases in *Spirulina* fed. *Spirulina* levels of 1% of the total ration during the week prior to slaughter has been shown to achieve broiler muscle tissue pigmentation best representing consumer preferences (Dismukes et al., 2008).

*Pigs*

Pig growth response to *Spirulina* is inconsistent. Hugh et al. (1985) found crossbred weanling pigs receiving *Spirulina* had 9% higher growth rates than control pigs. Paradoxically, Grinstead et al. (1998) observed no growth difference between *Spirulina*-fed and control pigs. However, this divergence is thought to be due to experimental methodology differences between these trials, particularly the compounding effect of crossbreeding and heterosis on growth. Other explanations revolve around dietary protein digestibility decreases with increased *Spirulina* levels found in pigs (Fevrier and, Seve, 1975). This results from *Spirulina's* complex structure resisting pig digestive enzymes which vary somewhat between pig breeds. As with chickens, it is assumed that *Spirulina's* effect on pig growth depends on the feed it replaces with *Spirulina* as a viable replacement for skim milk powder (Grinstead et al., 1998).

An advantage to pig producers feeding *Spirulina* is an increase in boar fertility. Granaci (2007a) found boars fed *Spirulina* extract had greater overall sperm quality compared to controls; with increased volume (11%), motility and post-storage viability (5%).

Rabbits

*Spirulina* has been trialled in commercially farmed meat rabbit feed rations. So far, its inclusion in rabbit diets has been shown not to influence growth (Peiretti and, Meineri, 2008) or carcass yields (Peiretti and, Meineri, 2011). These findings may quell concerns that feed rations containing *Spirulina* would be less digestible than conventional diets. However, rabbits fed *Spirulina* have an increased total feed consumption compared to control (Peiretti and, Meineri, 2008). Dietary *Spirulina* levels of 1% of total dry matter were found to improve crude protein digestibility in rabbits fed both low- and high-fat diets compared to controls (Peiretti and, Meineri, 2009). Hence, including *Spirulina* into rabbit diets may be useful when basal diets are

high in fat to provide sufficiency energy to 'fuel' optimal growth rates (Peiretti and, Meineri, 2009).

Rabbit meat quality has been shown to improve when *Spirulina* is fed. For instance, Meineri et al. (2009) and Peiretti and Meineri (2011) both identified *Spirulina* as a causal factor for increasing GLA and n-6/n-3 PUFA ratios within rabbit muscle FA profile. This supports continued consumer-preferred meat colour and appearance by improving rabbit meat's oxidative stability (Dalle Zotte and, Szendro, 2011). Furthermore, GLA has health benefits for humans (Howe et al., 2006), and its increased level in rabbit meat would appeal to health-conscious consumers. Rabbit health has also been found to improve with dietary *Spirulina*, as rabbits fed *Spirulina* had higher oxyhaemoglobin levels than controls (Meineri et al., 2009).

*Ruminants*

*Spirulina* trials using dairy cows have produced positive results with direct impact on productivity. Kulpys et al. (2009) found cows fed *Spirulina* had a 21% increase in their milk production. Furthermore, Simkus et al. (2007; 2008) showed increases in milk fat (between 17.6% and 25.0%), milk protein (9.7%), and lactose (11.7%) in cows fed *Spirulina* compared to controls. Milk SFA content decreased and UFA increased with *Spirulina* consumption (Christaki *et al.*, 2012). These results could be attributable to *Spirulina's* influence on microbial protein synthesis, avoidance of rumen degradation and its nutrient-rich composition. Moreover, these findings highlight *Spirulina's* use in enhancing milk's health appeal.

*Spirulina* has been associated with significant decreases in milk somatic cell count (Simkus et al., 2007), thus improving milk's food safety value. Additionally, dairy cows fed *Spirulina* have been found to have improved body condition (between 8.5% and 11%) compared to controls (Kulpys et al., 2009).

As shown with boars, bull sperm quality is improved with *Spirulina*. Sperm motility, concentration and post-storage viability were all positively affected when bulls

received a bio-extract removed from *Spirulina* (Granaci, 2007b). However, the effect of 'raw' dietary *Spirulina* on bull sperm quality need to be further studied.

To the best of our knowledge, the effect of *Spirulina* on sheep products and productivity remains relatively unexplored. Only two published studies have been found:

1. Bezerra et al. (2010) found that lambs fed *Spirulina* enriched milk had higher liveweights and ADG than controls
2. Shimkiene et al. (2010) showed that pregnant ewes fed *Spirulina* deliver heavier lambs (up to 4.07%) with greater ADG compared to controls

**Summary**

Australian lamb producers are shifting away from singular market focus to dual-purpose production systems. These inherently mate meat-type rams over a core purebred Merino flock to ensure progeny have desirable wool and meat characteristics. Consequently, Australian producers can maintain interests in the declining wool market and the highly profitable and expanding meat market. This bifocal approach relies on optimising product quality and productivity.

Wool is intrinsically a fibrous product predominately comprised of sulphur-rich keratin proteins. These confer unique wool characteristics to each fibre, being; rigidity, strength, durability and processing performance. Wool quality is routinely assessed in terms of physical fibre characteristics which permit accurate price and end use discrimination of the raw product. Insight into the proteome of wool is thought to also contribute to quantifying quality albeit presently under-researched.

Meat, or muscle tissue, is the primary consumable lamb product and increased quantity or yield is preferable. This can be objectively monitored as liveweight, body conformation and growth rate. Haematological traits can provide insight into overall lamb health and response to environmental changes, such as nutrition. However,

meat yield is not the sole determinant of value with sensory and nutritional quality contributing. Sensory quality refers to consumer response upon maceration whereas nutritional quality to human health benefits. Both qualities can be assessed by fatty acid profile scrutiny.

Dual-purpose producers can manipulate lamb product qualities using sire breed and its interaction with sex as genetic tools for selection. Similarly, lamb response to dietary supplementation differs as rumen microbes alter final lipid, carbohydrate and fatty acid content and composition. Therefore, supplementation is routinely employed to alter lamb product quality, productivity and feed intake.

*Spirulina* is a relatively recently recognised protein-rich supplement in livestock industries. This application is buoyed by its nutritional composition and its association with improved productivity and product quality in chickens, pigs, rabbits and cattle. Yet, *Spirulina's* application in lamb production and its effect on productivity and product quality remains largely unknown due to the current paucity in published information. Moreover, *Spirulina's* unknown relationship with dual-purpose lamb production methods in Australia, such as crossbreeding, supplementation level, and gender interactions contributes to the significance of this void in current knowledge.

To develop a complete understanding of *Spirulina's* applications in Australian dual-purpose production systems, a study into its effect and interactions with lamb sire breed, sex and supplementation level would prove beneficial. The outcomes of this study would assist future dual-purpose lamb producers to best utilise *Spirulina* as a protein-rich supplement to manage productivity and product quality.

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Chapter 2

# Effect of *Spirulina* supplementation on wool quality change in dual-purpose lambs under typical pasture-based management and simulated-drought

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**ABSTRACT**

Forty-eight purebred and crossbred Merino $F_1$ lambs sired by White Suffolk, Dorset, Merino and Black Suffolk lambs were supplemented with *Arthrospira platensis* (*Spirulina*) under typical pasture-based and simulated drought conditions over two consecutive years. The objective was to investigate the effect of supplementation level, basal diet, sire breed and sex as well as their interactions on wool quality parameters. We tested the hypothesis that *Spirulina* supplementation will improve wool yield without compromising wool quality in these lambs. A complete randomized experimental design, balanced by 4 sire breeds, 3 supplementation levels and 2 sexes was utilised. Lambs were randomly allocated into 3 treatments (8 lambs per treatment) – the CONTROL group grazing without *Spirulina* (0%), LOW (50ml), MEDIUM (100 ml) and HIGH (200 ml) levels. All lambs had *ad libitum* access to the basal diet of either ryegrass pastures (typical) or lucerne hay (drought) and barley. Lambs in the LOW and HIGH supplementation groups were individually orally drenched daily with *Spirulina* solution prior to either being released with the CONTROL group of lambs into paddocks for grazing or fed Lucerne hay in metabolic crates over a 42-day period after a 21-day adjustment phase. Mid-side wool was sampled at the beginning and end of the 63-day experimental period and commercially assessed by the Australian Wool Testing Authority for wool fibre diameter (FD), coefficient of variation (CV), standard deviation (SD), spinning

fineness (SF), comfort factor (CF), fibre curvature (CURV), and clean fleece yield (YIELD). Data were transformed as wool trait changes (Δ) from the beginning to end of the feeding trial and analysed in SAS with sire breed, *Spirulina* supplementation level, basal diet, sex, sampling date, and their second order interactions as fixed effects, sire as a random effect and wool quality traits as dependent variables. Wool ΔYIELD was significantly influenced by level of supplementation, type of basal diet and sex. *Spirulina*-supplemented lambs during drought conditions exhibited higher ΔYIELD responses that seemed to increase with the level of supplementation. Unsupplemented lambs had the worst ΔYIELD regardless of basal diet or supplementation. Sire breed and sex variations were significant ($P<0.05$) as wool from Suffolk-sired lambs and ewes had the highest ΔCV and ΔYIELD, respectively. ΔCV and ΔSD were the most affected wool traits by significant interactions between *Spirulina* supplementation level, sire breed and sex. It was concluded that *Spirulina* supplementation during drought improves wool ΔYIELD without compromising wool fibre diameter, comfort factor and spinning fineness in dual-purpose prime lambs.

(**Keywords**: *Arthrospira platensis*, protein supplementation, wool, sheep, dual-purpose)

**Introduction**

*Spirulina* (*Arthrospira platensis*) is a highly nutritious, protein-rich and edible cyanobacterium. A review of literature on its use as a supplementary livestock feed has been published (Holman and, Malau-Aduli, 2012b). However, to the best of our current knowledge, research into *Spirulina's* potential for supplementing dual-purpose prime lambs where wool and meat production are of immense economic interests, remains scarce. Apart from the influence of *Spirulina* on growth and body conformation traits (Holman *et al.*, 2012), there is no published literature on *Spirulina's* impact on wool quality traits in prime lambs, thus creating a knowledge gap that needs to be filled.

The sheep industry's use of crossbreeding and protein-rich supplements in dual-purpose prime lamb operations as tools to boost lamb growth rates and liveweight gains (ABARE., 2012) could potentially be complemented by *Spirulina's* protein-rich nature. This is as the availability of many traditional supplements is projected to decline in the short to medium-term due to increasing competition from other sectors such as biofuels, consumables, urban sprawl land encroachment and climatic events (Harle et al., 2007; Nardone et al., 2010). Therefore, alternative protein-rich supplements such as *Spirulina*, are essential to future industry stability and development (Hume *et al.*, 2011).

Apart from meat production, dual-purpose lamb operations derive a significant proportion of their economic returns from wool production. However, crossbreeding and supplementation with traditional protein-rich sources such as grains and canola, have been associated with lesser wool quality (Malau-Aduli and, Akuoch, 2012; Masters et al., 1999). Wool quality traits are objectively assessed routinely in the Australian sheep industry because of links to intrinsic economic value and textile manufacturing performances (Holman and, Malau-Aduli, 2012a).However, these traits can be influenced by crossbreeding, type and level of protein-rich supplementation, lamb sire breed, sex and basal diet. Hence, the impact of these factors on wool quality must be included in any research investigation into alternative protein-rich sources of dietary supplementation in prime lambs.

Our aim was to evaluate the effect of *Spirulina* supplementation and its interactions with sire breed, sex and basal diet on wool quality in purebred and crossbred Merino lambs under typical pasture-based and simulated-drought conditions over two consecutive years. We hypothesised that *Spirulina* supplementation will improve wool yield without compromising wool quality in dual-purpose prime lambs. .

**Materials and methods**

This experiment was carried out at the University of Tasmania (UTAS) Farm, Cambridge, Hobart, Tasmania, Australia, in accordance with the 1993 Tasmanian

Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004) and approved by the UTAS Animal Ethics Committee.

*Animal management and experimental design*

Over two consecutive years, a mating ratio of 1 terminal ram to 100 Merino ewes was used to produce approximately 1600 F1 crossbred progeny. At 12 weeks of age, all progeny were identified using National Livestock Identification ear tags and weaned onto ryegrass pastures. At 6 months old, a total of 48 lambs was randomly selected for each feeding trial; 24 lambs on typical ryegrass pasture basal diet (Year 1); and, 24 lambs on simulated-drought basal diet of lucerne hay (Year 2). Each feeding trial continued for 9-weeks after three weeks of adjustment, during which commercially purchased *Spirulina* powder (TAAU, Darwin, Northern Territory, AUS) was directly supplemented to lambs by oral drenching daily in a water suspension at a *Spirulina* (g) : Water (mL) ratio of 1:10 w/v.

*Typical basal diet*: Random lamb allocation into the following treatment groups was undertaken: CONTROL (0 mL), MEDIUM (100 mL) and HIGH (200 mL) *Spirulina* supplementation levels balanced by lamb sire breed (Black Suffolk, Dorset, Merino and White Suffolk) and sex (ewes and wethers). All lambs had *ad libitum* access to drinking water, 150 g of barley grains per day and were run as a single flock on ryegrass pasture.

*Simulated-drought basal diet*: Treatment groups of *Spirulina* supplementation levels were: CONTROL (0 mL), LOW (50 mL), MEDIUM (100 mL), and HIGH (200 mL); sire breeds – Dorset, Merino and White Suffolk; and sex – ewes and wethers. Lambs were confined in individual 0.6 m x 1.2 m metabolic crates with *ad libitum* access to drinking water and Lucerne hay, which was replaced daily. All lambs received barley (150 g/day).

*Wool sampling and analysis*

Midside wool samples of approximately 10 $cm^2$ were shorn using Oster-Sunbeam electric shears, from the same location on each lamb at the commencement and completion of each feeding trial as per Baxter and Cottle (1998). These samples were accurately catalogued and analysed using the Sirolan Laserscan™ (CSIRO, Melbourne, AUS) at the Australian Wool Testing Authority (AWTA, Melbourne AUS) for clean fleece yield (YIELD), mean fibre diameter (FD), coefficient of fibre diameter variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), fibre curvature (CURV) and spinning fineness (SF).

*Chemical analysis of Spirulina and basal diet components*

Dry matter content of *Spirulina* and the basal diets was determined by drying samples to a constant weight at 65°C in a fan forced oven. Ash content was determined by combusting samples in a furnace at 550°C for 5 hours. Neutral detergent fibre and acid detergent fibre contents were measured using an Ankom fibre analyser (ANKOM220; Ankom Technology USA) (van Soest *et al.*, 1991). Total N content was measured using the Kjeldahl method (van Soest et al., 1991) and multiplied by 6.25 to find crude protein values. Ether extract was determined using Soxhlet methodology of fat extraction while in vitro digestibility and metabolisable energy was estimated using near infrared reflectance spectroscopy (Garnsworthy and, Unal, 2004).

**Table 2.1** Nutrient composition (g/100g DM) and dry matter content (g/100g fresh wt) of *Spirulina* and basal diet of ryegrass pasture and barley grain[1]

| *Chemical composition* | **Feed components** | | | |
|---|---|---|---|---|
| | *Spirulina* | Barley grain | Ryegrass pasture | Lucerne hay |
| DM | 96.0 | 93.2 | 44.7 | 90.6 |
| NDF | 32.6 | 18.5 | 22.4 | 36.0 |
| NDFn[2] | 30.3 | 17.2 | 20.8 | 33.5 |
| ADF | 18.3 | 6.0 | 23.0 | 29.0 |
| NFC[3] | 7.9 | 68.7 | 43.5 | 35.2 |
| Ash | 9.5 | 3.2 | 11.9 | 6.9 |
| EE | 5.9 | 2.0 | 3.0 | 1.9 |
| CP | 62.2 | 8.9 | 20.8 | 22.5 |
| ME, kJ/100g DM[4] | 580.4 | 307.0 | 381.3 | 564.3 |

[1] Dry matter (DM), neutral detergent fibre (NDF), nitrogen free NDF (NDFn), non-fibrous carbohydrate (NFC), acid detergent fibre (ADF), ether extract (EE), indigestible organic matter (IOM), and metabolisable energy (ME)
[2] NDFn = NDF x 0.93 (Undersander and, Moore, 2002)
[3] NFC = 100 – (NDFn + CP + EE + Ash) (Undersander and, Moore, 2002)
[4] ME = 0.0148(NFD) + 0.0166(EE) (Moss and Givens, 1994)

*Statistical analysis*

Prior to analysis, all data were transformed to assess the change in wool traits (Δ) over the duration of the feeding trial by computing the difference between the initial and final values for each of the wool quality traits. The 'Statistical Analysis System' software package (SAS Institute., 2009) was utilised to compute unadjusted means, standard deviations, minimum, maximum values and a range of summary statistics that were carefully examined for any data entry errors or outliers. ANOVA by PROC GLM analysis (SAS Institute., 2009) was run fitting *Spirulina* supplementation level, sire breed, sex, basal diet and their interactions as fixed effects in the model and wool traits as dependent variables. Duncan's multiple range and Bonferroni's probability pairwise comparison tests (SAS Institute., 2009) were used to separate means at the $P<0.05$ threshold of statistical significance.

**Results**

Lambs in the HIGH and LOW *Spirulina* supplementation levels had higher wool ΔYIELD than CONTROL lambs and ewe lambs recorded the highest ΔYIELD (Table 2.2). Lambs fed on the simulated-drought basal diet had higher wool ΔYIELD than their

counterparts on typical pasture-based basal diets. Black Suffolk-sired lambs had the highest ΔCV compared with other sire breeds. No other independent effects on wool quality traits were found (*P*>0.05; Table 2.2).

**Table 2.2a** Continuation of Table 2.2b describing level of significance (*P* values) of changes in physical wool traits [1, 2]

| | *P values* | | | | | | |
|---|---|---|---|---|---|---|---|
| | S | B | G | D | S x B | S x G | S x D |
| ΔYIELD (%) | 0.020 | 0.389 | 0.044 | 0.001 | 0.210 | 0.057 | 0.633 |
| ΔFD (µm) | 0.708 | 0.573 | 0.564 | 0.381 | 0.720 | 0.434 | 0.706 |
| ΔCV (%) | 0.674 | 0.063 | 0.063 | 0.827 | 0.203 | 0.172 | 0.478 |
| ΔSD | 0.868 | 0.355 | 0.217 | 0.663 | 0.381 | 0.173 | 0.811 |
| ΔCF (%) | 0.848 | 0.431 | 0.967 | 0.333 | 0.718 | 0.626 | 0.923 |
| ΔCURV (°/mm) | 0.919 | 0.396 | 0.985 | 0.867 | 0.253 | 0.203 | 0.469 |
| ΔSF (µm) | 0.494 | 0.198 | 0.658 | 0.069 | 0.822 | 0.814 | 0.846 |

[1] *Spirulina* supplementation level (S), sire breed (B), sex (G), basal diet (D).
[2] Clean fleece yield (YIELD), mean fibre diameter (FD), fibre diameter standard deviation (SD), fibre diameter coefficient of variation (CV), comfort factor (CF), fibre curvature (CURV), and spinning fineness (SF).

**Table 2.2b** Effects of *Spirulina* supplementation level, sire breed, sex and basal diet on changes in physical wool traits [1, 2]

| | ***Spirulina* supplementation level (S)** | | | | **Sire breed (B)** | | | | **Sex (G)** | | **Basal diet (D)** | | *RMSE* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CONTROL | LOW | MEDIUM | HIGH | Black Suffolk | Dorset | Merino | White Suffolk | Ewe | Wether | Typical | Simulated-drought | |
| ΔYIELD (%) | -2.84 | 2.35 | -1.51 | 0.74 | -3.07 | -0.98 | -0.83 | 0.52 | 0.21 | -1.73 | -4.39 | 2.88 | 2.99 |
| ΔFD (µm) | 0.41 | 1.57 | 0.61 | 0.33 | -0.70 | 1.07 | 0.39 | 0.86 | 1.08 | 0.10 | 0.60 | 0.58 | 2.62 |
| ΔCV (%) | 1.09 | -0.97 | 0.55 | -0.11 | 3.03 | -0.04 | -0.71 | 0.56 | 0.45 | 0.20 | 0.76 | -0.11 | 2.42 |
| ΔSD | 0.34 | 0.07 | 0.23 | 0.06 | 0.65 | 0.19 | -0.07 | 0.26 | 0.33 | 0.06 | 0.30 | 0.08 | 0.64 |
| ΔCF (%) | -4.75 | -3.85 | -4.29 | -2.46 | 1.17 | -5.33 | -2.80 | 5.52 | -4.35 | -3.32 | -4.48 | -3.20 | 10.21 |
| ΔCURV (°/mm) | -3.57 | -1.83 | -2.21 | -5.00 | -5.00 | -5.71 | 1.57 | -5.29 | -3.38 | -3.38 | -3.96 | -2.79 | 8.86 |
| ΔSF (µm) | 1.44 | 1.35 | 0.70 | 0.34 | -0.02 | 1.86 | 0.28 | 0.93 | 1.15 | 0.63 | 1.24 | 0.55 | 2.15 |

[1] Clean fleece yield (YIELD), mean fibre diameter (FD), fibre diameter standard deviation (SD), fibre diameter coefficient of variation (CV), comfort factor (CF), fibre curvature (CURV), and spinning fineness (SF).
[2] Root mean square error (RMSE).

However, there were significant sire breed, supplementation level and sex interactions that influenced wool quality traits (Figures 2.1-3). At LOW levels of *Spirulina* supplementation, the wool ΔCV of White Suffolk-sired lambs was lower than that of CONTROL (unsupplemented) lambs (Figure 1; Table 2.3). Wether lambs receiving MEDIUM levels of *Spirulina* supplementation had higher ΔCV than lambs supplemented at LOW *Spirulina* levels (Figure 2.2; Table 2.4). Dorset-sired lambs had the highest ΔSD, followed sequentially by White Suffolk- and Merino-sired lambs on simulated-drought basal diet, but on typical pasture-based basal diet, Black Suffolk-sired lambs had higher ΔSD than Dorset-sired lambs (Figure 2.3a). Other significant second order interactions were observed in which Dorset-sired lambs fed typical pasture-based basal diets had the highest ΔCV in comparison with lambs on simulated-drought basal diet. When lambs were on typical pasture-based diets, it was observed that the ΔCV of Black Suffolk-sired lambs were higher than that of Dorset-sired lambs (Figure 2.3b).

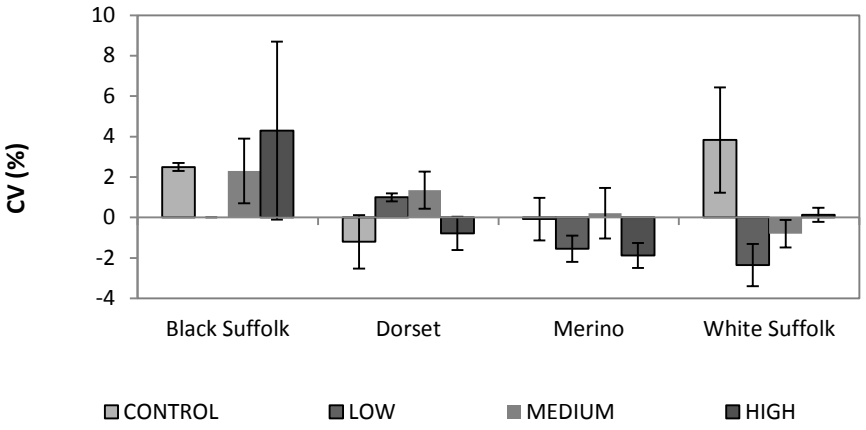


**Figure 2.1** *Spirulina* supplementation level and sire breed interaction on ΔCV – changes in coefficient of fibre diameter variation ($P$<0.029).

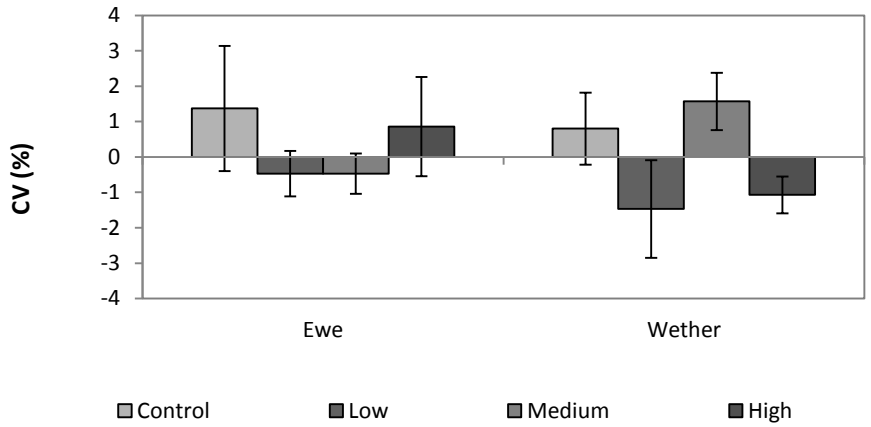


**Figure 2.2** *Spirulina* supplementation level and sex interaction with ΔCV – changes in coefficient of fibre diameter variation (*P*<0.024).

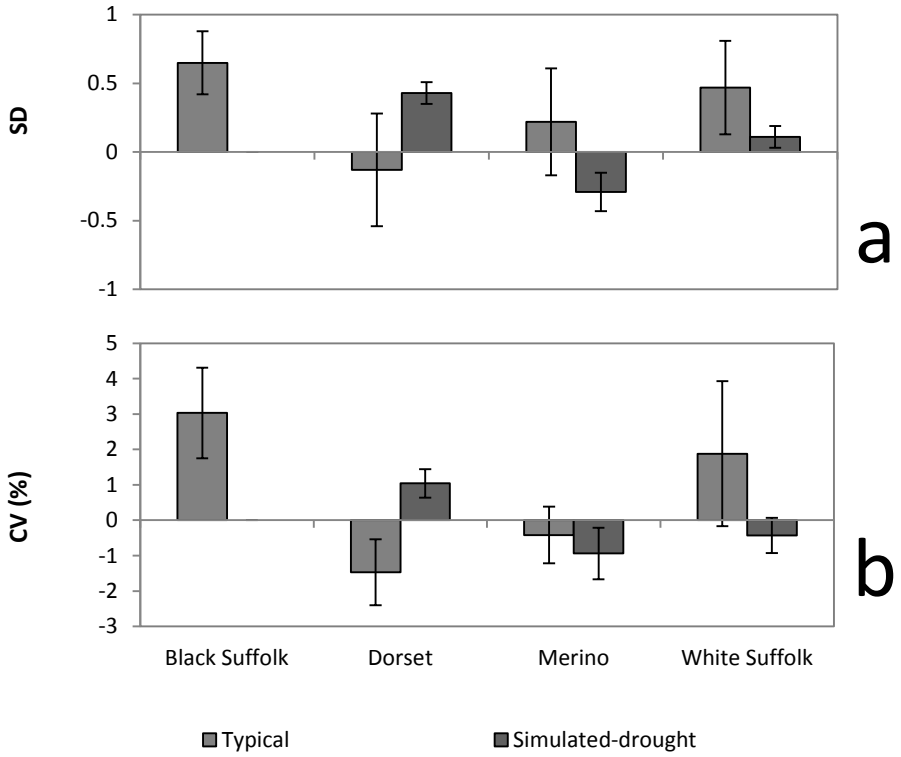


**Figure 2.3** Basal diet and sire breed interactions with a) ΔSD – changes in fibre diameter standard deviation (*P*<0.026); and b) ΔCV – changes in coefficient of fibre diameter variation (*P*<0.021).

**Table 2.3** Effects of *Spirulina* supplementation level (CONTROL, LOW, MEDIUM, HIGH) and sire breed interactions on changes in physical wool traits, with level of significance (P values) shown on Table 2b [1]

| | ΔFD (µm) | ΔCV (%) | ΔSD | ΔCF (%) | ΔSF (µm) | ΔCURV (°/mm) | ΔYIELD (%) |
|---|---|---|---|---|---|---|---|
| **CONTROL** | | | | | | | |
| Black Suffolk | -0.05 | 2.50 | 0.60 | -1.75 | 0.45 | -12.00 | -6.95 |
| Dorset | 0.17 | -1.20 | -0.23 | -3.03 | 2.88 | 1.50 | -3.50 |
| Merino | 1.80 | -0.08 | 0.30 | -10.13 | 1.70 | 0.50 | -1.48 |
| White Suffolk | -0.53 | 3.83 | 0.80 | -2.60 | 0.23 | -8.50 | -1.48 |
| **LOW** | | | | | | | |
| Black Suffolk | . | . | . | . | . | . | . |
| Dorset | 1.15 | 1.00 | 0.45 | -4.90 | 1.25 | -9.00 | 1.50 |
| Merino | 0.90 | -1.55 | -0.15 | 0.25 | 0.70 | -1.00 | 3.95 |
| White Suffolk | 2.65 | -2.35 | -0.10 | -6.90 | 2.10 | 4.50 | 1.60 |
| **MEDIUM** | | | | | | | |
| Black Suffolk | 0.05 | 2.30 | 0.60 | -3.75 | 0.55 | -0.50 | -2.80 |
| Dorset | 1.40 | 1.35 | 0.58 | -8.83 | 1.60 | -10.25 | -0.98 |
| Merino | -0.07 | 0.22 | 0.00 | 0.03 | -0.08 | 4.50 | -4.78 |
| White Suffolk | 0.80 | -0.80 | -0.08 | -4.33 | 0.65 | -1.75 | 1.87 |
| **HIGH** | | | | | | | |
| Black Suffolk | -2.10 | 4.30 | 0.75 | 9.00 | -1.05 | -2.50 | 0.55 |
| Dorset | 1.60 | -0.78 | 0.08 | -4.35 | 1.40 | -6.75 | 0.30 |
| Merino | -0.80 ± 0.75 | -1.88 | -0.48 | 0.18 | -1.00 | 1.00 | 1.38 |
| White Suffolk | 1.40 ± 0.36 | 0.13 | 0.25 | -8.95 | 1.33 | -10.50 | 0.63 |

[1] Clean fleece yield (YIELD), mean fibre diameter (FD), fibre diameter standard deviation (SD), fibre diameter coefficient of variation (CV), comfort factor (CF), fibre curvature (CURV), and spinning fineness (SF).

**Table 2.4** Effects of *Spirulina* supplementation level (CONTROL, LOW, MEDIUM, HIGH) and sex interactions on changes in physical wool traits, with level of significance (P values) shown on Table 2b [1]

| | CONTROL | | LOW | | MEDIUM | | HIGH | |
|---|---|---|---|---|---|---|---|---|
| | **Ewe** | **Wether** | **Ewe** | **Wether** | **Ewe** | **Wether** | **Ewe** | **Wether** |
| ΔFD (μm) | -0.93 | -0.47 | 1.54 | 0.60 | 2.06 | -1.27 | 0.49 | 1.23 |
| ΔCV (%) | 3.90 | 2.17 | -0.20 | 0.13 | -1.41 | -0.01 | 1.50 | -0.37 |
| ΔSD | 0.83 | 0.47 | 0.23 | 0.14 | 0.11 | -0.26 | 0.41 | 0.11 |
| ΔCF (%) | 1.20 | 1.13 | -6.16 | -4.50 | -5.71 | 0.11 | -3.57 | -7.47 |
| ΔSF (μm) | -0.03 | 0.00 | 1.43 | 2.29 | 1.77 | -1.21 | 0.76 | 1.10 |
| ΔCURV (°/mm) | -2.33 | -7.67 | -4.43 | -7.00 | 1.00 | 2.14 | -7.14 | -3.43 |
| ΔYIELD (%) | -1.27 | -4.87 | 0.41 | -2.37 | 0.07 | -1.73 | 0.79 | 0.26 |

[1] Clean fleece yield (YIELD), mean fibre diameter (FD), fibre diameter standard deviation (SD), fibre diameter coefficient of variation (CV), comfort factor (CF), fibre curvature (CURV), and spinning fineness (SF).

In terms of sex and supplementation level interactions, ewe lambs fed typical pasture-based basal diets and supplemented on LOW level of *Spirulina* supplementation had lower ΔSD than unsupplemented (CONTROL) lambs (Figure 2.4a). On the other hand, CONTROL ewe lambs fed typical pasture-based basal diets had higher ΔCV than lambs supplemented at both LOW and MEDIUM *Spirulina* levels. Wethers fed typical pasture-based basal diets and ewes fed simulated-drought basal diets had lower ΔCV at HIGH *Spirulina* supplementation level than other lambs on MEDIUM supplementation (Figure 2.4b).

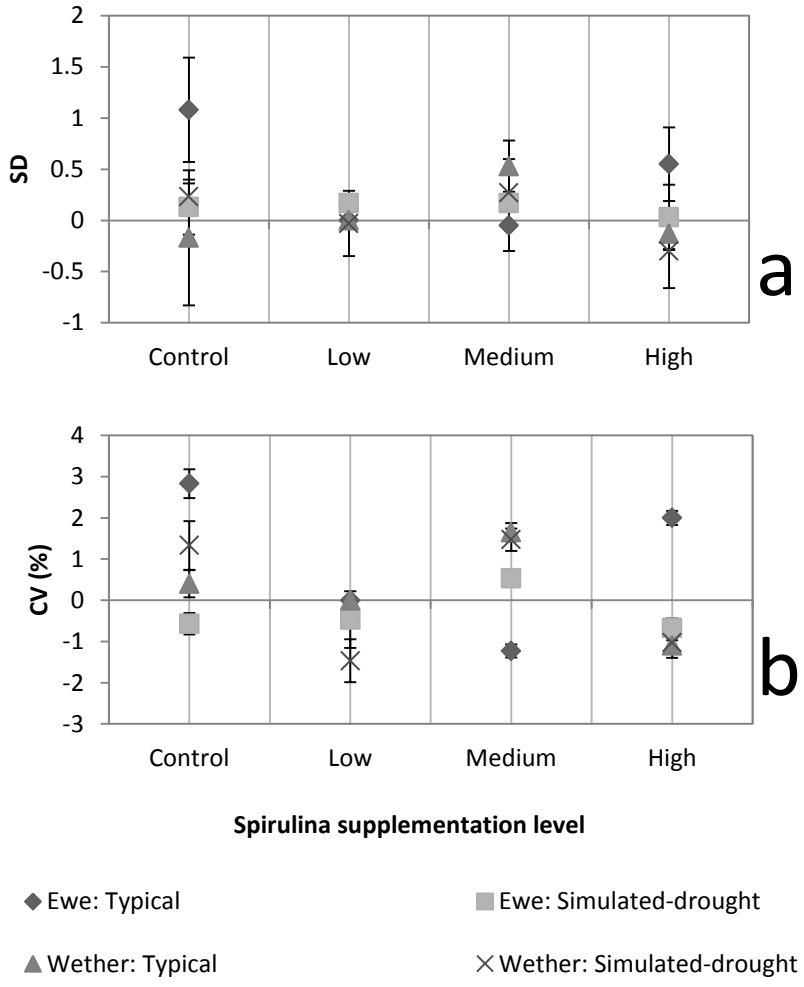


**Figure 2.4** *Spirulina* supplementation level, sex, and basal diet interactions with a) ΔSD – changes in fibre diameter standard deviation ($P$<0.012); and b) ΔCV – changes in coefficient of fibre diameter variation ($P$<0.043).

**Discussion**

From the early review of *Spirulina's* potential use as an animal feed by Belay et al. (1996) to the current updated literature on its use as a livestock supplement (Holman and, Malau-Aduli, 2012b), there is no published information on the influence of *Spirulina* supplementation on the wool quality of genetically divergent prime lambs. Therefore, to fill this knowledge gap, this study tested the hypothesis that *Spirulina supplementation will improve wool yield without compromising wool quality in dual-purpose prime lambs*.

YIELD refers to the fibrous content of wool and attracts high price premiums (Holman and, Malau-Aduli, 2012a; Mortimer et al., 2010). Wool fibres are primarily composed of proteins (Plowman, 2003), hence it is possible that with increasing supplementation levels of protein-rich *Spirulina*, there was a corresponding increase in nutrient partitioning towards wool fibre synthesis, hence the observed increase in YIELD. This is in agreement with published literature demonstrating direct correlated response of wool YIELD to increased dietary protein (Friend and, Robards, 2005; Slen and, Whiting, 1952).

Wool synthesis is limited by the availability of sulphur amino acids – cysteine and methionine which are known to increase wool fineness due to low FD (Hynd and, Masters, 2002; Liu and, Masters, 2003). However, *Spirulina* is low in sulphur amino acid content compared to other protein-rich supplements (Ciferri and, Tiboni, 1985; Volkmann et al., 2008). This low S-amino acid content could have been a contributing factor to FD remaining unchanged with increase in *Spirulina* supplementation observed in this study. This suggests that protein sourced from *Spirulina* and partitioned towards wool fibre synthesis promotes fibre growth rate and yield without influencing with fibre diameter. Habib *et al*. (2001) reported that YIELD increased with dietary protein levels while FD remained unchanged.

The variation in YIELD due to differences in basal diet could have three possible explanations; 1) The efficiency of feed conversion to wool increased with decreased

feed levels (Naqvi and, Rai, 1990) due to changes in energy partitioning; 2) Differences between typical and simulated-drought basal diets in protein degradability and sulphur amino acid content have been identified as two predominant causal factors of YIELD differences between diets (White *et al.*, 2000); and 3) simulated-drought lambs were not exposed to wool contaminants such as dust and vegetable matter typical of pasture fed lambs which are known to decrease YIELD, (Holman and, Malau-Aduli, 2012a). As, sheep sheltered from wool contaminants using jackets, have previously been shown to have improved YIELD (Hatcher *et al.*, 2008).

Entire fleece fibre diameter variation is measured objectively using either SD or CV, with CV being a refinement of SD (Holman and, Malau-Aduli, 2012a). While low SD and high CV fleece fibre diameter is preferable, there is currently little or no price incentive (Holman and, Malau-Aduli, 2012a). The significant interactions between sire breed and supplementation level on SD and CV is thought to arise from the strong influence of the genetic make-up of lambs on nutrient partitioning pathways towards wool synthesis (Li *et al.*, 2008; Scales et al., 2000). Genetic potential varies with lamb sire breed and sex; and have been shown to interact with lambs' diet to affect wool synthesis and wool quality traits (Kellaway, 1973; Malau-Aduli and, Akuoch, 2012; Malau-Aduli et al., 2012b; Pitchford, 1992; Scales et al., 2000). Nevertheless, only SD and CV in White Suffolk-sired lambs and wethers were affected by these variations. Therefore, it is most likely that the common Merino maternal genetics of all the lambs used in this study 'standardised' any changes in wool traits over the entire feeding trial period.

**Conclusions**

Dual-purpose and prime lamb operations can benefit from our main finding that clean fleece yields is improved with *Spirulina* supplementation without compromising other wool quality traits such as fibre diameter, wool comfort factor and spinning fineness. Furthermore, the observed interaction effects of basal diet, sire breed and sex with *Spirulina* supplementation levels permit flexibility in

operational options of optimising profitability from wool in the prime lamb industry. Further research to give a greater understanding of internal nutrient partitioning pathways and controlling factors of *Spirulina* supplementation effects on lamb growth and liveweight is needed due to their major contribution to profitability in dual-purpose prime lamb operations.

**Acknowledgements**

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Chapter 3

# Wool quality traits of grazing purebred and crossbred Merino lambs orally drenched with *Spirulina*

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**ABSTRACT**

We tested the hypothesis that *Spirulina* supplementation will not be detrimental to lamb wool quality. Our experimental objective was to evaluate the effect of *Spirulina* supplementation on the wool characteristics of grazing purebred and crossbred Merino weaned lambs under a single pasture-based management system and their interaction with sire breed and sex. A complete randomized block experimental design, balanced by 4 sire breeds, 3 supplementation levels and 2 sexes (4 x 3 x 2). The weaner ewe and wether lambs from Merino, White Suffolk, Dorset, Black Suffolk sire breeds were randomly allocated into 3 treatments (8 lambs per treatment) – the CONTROL group grazing without *Spirulina* (0%), MEDIUM (100ml) and HIGH (200ml) *Spirulina*. All lambs had *ad libitum* access to the basal diet of ryegrass pastures and crushed barley. The *Spirulina* powder was dissolved in water utilising a weight (g): volume (mL) ratio of 1:10 (MEDIUM) or 2:20 (HIGH) and the lambs orally drenched daily for nine weeks. Both CONTROL and *Spirulina* supplemented group of lambs were kept in a single mob. Lambs in the MEDIUM and HIGH *Spirulina* treatment groups were individually drenched daily with *Spirulina* solution prior to being released with the control group of lambs into paddocks for grazing over a 6-week period following a 3-week adjustment phase. Mid-side wool was sampled at the beginning and end of the 9-week period and commercially assessed by the Australian Wool Testing Authority for wool fibre diameter (FD), coefficient of variation (CV),

standard deviation (SD), spinning fineness (SF), comfort factor (CF), fibre curvature (CURV), and clean fleece yield (YIELD). Data were analysed in SAS using MIXED linear model procedures, with sire breed, *Spirulina* supplementation level, sex, sampling date, and their second order interactions as fixed effects, sire as a random effect and wool quality traits as dependent variables. *Spirulina* supplementation had no effect on wool characteristics as differences between the CONTROL and supplemented groups were not significant ($P>0.05$). However, sire breed significantly ($P<0.001$) influenced FD, SF, CF and CURV with purebred Merinos having superior wool quality than crossbreds. Wethers grew higher quality wool than ewes. These findings highlight *Spirulina's* promising potential as an alternative supplementary feed, especially in dual-purpose sheep production systems, because it does not compromise wool quality in supplemented weaner lambs, hence the acceptance of our hypothesis.

(**Keywords**: *Spirulina, wool quality, Merino crossbreds, supplementation, dual-purpose*)

**Introduction**

*Spirulina* (*Arthrospira platensis*) is an edible cyanobacterium with potential as an alternative protein-rich lamb supplement due to its high protein content of 60-70% (Becker, 2007). *Spirulina* is also rich in essential vitamins, minerals, fatty acids, amino acids and carotenoids. Supplementary feeding with *Spirulina* has been previously tried with several animal species (Holman and, Malau-Aduli, 2012b), but to the best of our knowledge, there is presently no published information on the impact of *Spirulina* supplementation on wool quality traits in dual purpose lambs.

Across Australia, farmers have adopted dual purpose sheep systems with both wool and meat production goals (Rowe, 2010). This objective is generally achieved by mating meat-type rams to a core flock of purebred Merino ewes and exploiting heterosis to optimise lamb growth. Current lamb meat prices are high (Martin and, Phillips, 2011), while wool prices are at a historical low (Gibbon and, Nolan, 2011).

Hence, profitability in dual purpose sheep systems is now mostly driven by lamb growth and early attainment of slaughter weight.

Lamb growth is commonly enhanced through protein-rich supplementation using canola meal, lupins, barley and wheat. However, varying and inconsistent reports on the impact of these supplements on wool quality have been reported (Malau-Aduli and, Akuoch, 2012; Masters and, Mata, 1996). Any decline in wool quality due to supplementary feeding can affect total farm profitability. Therefore, we tested the hypothesis that *the oral drenching of grazing purebred Merino and crossbred lambs with Spirulina supplement will not elicit a significant variation and decline in wool quality*. The experimental objective was to evaluate the effect of *Spirulina* supplementation and interactions with sire breed and sex on wool quality traits in purebred and crossbred Merino lambs under a single pasture-based management.

**Materials and Methods**

This study was conducted at the University of Tasmania Farm, Cambridge, Tasmania, Australia. All procedures had the University of Tasmania Animal Ethics approval and were conducted in accordance with the 1993 Tasmania Animal Welfare Act and the 2004 Australian code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004).

*Animal management and experimental design*

Approximately 1600 purebred Merino ewes were mated to 16 terminal sires at a mating ratio of 1 ram to 100 ewes in separate paddocks. All lambs were properly identified with their National Livestock Identification electronic ear tags that could be scanned automatically by the walk-over weighing scale. Five hundred of the progeny were raised on ryegrass pastures until weaning at 12 weeks of age. For the supplementation trial, a 4 x 3 x 2 factorial experimental design in which 24 weaner lambs with an average liveweight of 37.6 ± 5.2 kg and body condition score of 3.1 ± 0.4 at 6 months of age was utilised. Lambs were balanced by sire breed (Merino,

White Suffolk, Dorset, Black Suffolk), gender (ewes, wethers) and randomly allocated into 3 treatments (8 lambs per treatment) – the CONTROL group grazing without *Spirulina* (0%), LOW (100ml) and HIGH (200ml) *Spirulina*. The *Spirulina* powder was dissolved in water utilising a weight:volume ratio of 1g:10ml (LOW) and 2g:20ml (HIGH) and the solution delivered to the lambs by oral drenching. Both CONTROL and *Spirulina* supplemented group of lambs were kept in a single mob and had *ad libitum* access to the basal diet of ryegrass pastures and crushed barley whose nutrient composition is depicted in Table 3.1. Lambs in the LOW and HIGH *Spirulina* treatment groups were individually drenched daily with *Spirulina* solution prior to being released with the control group of lambs into paddocks sown with ryegrass pastures. The lambs were allowed 3 weeks of adjustment to the *Spirulina* drench prior to the experimental phase lasting 6 weeks. At all times, lambs had *ad libitum* access to clean drinking water.

*Wool sampling and analysis*

Midside wool samples of approximately $10cm^2$ were shorn from each lamb by an experienced shearer using Oster-Sunbeam electric shears (Baxter and, Cottle, 1998) at the start and completion of the feeding trial. Samples were accurately catalogued and commercially analysed at the Australian Wool Testing Authority (Melbourne, Australia) using LaserScan equipment (Heath *et al.*, 2006). The wool quality traits assessed were; mean fibre diameter (FD) using LaserScan OFDA, standard deviation (SD), coefficient of variation (CV), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV) and clean fleece yield (YIELD).

*Chemical analysis of the basal diet*

Dry matter content of the basal diet was determined by drying samples to a constant weight at 65°C in a fan forced oven. Ash content was determined by combusting samples in a furnace at 550°C for 5 hours. Neutral detergent fibre and acid detergent fibre contents were measured using an Ankom fibre analyser (ANKOM 220; Ankom Technology, NY, USA) (van Soest et al., 1991). Total N content was measured using

the Kjeldahl method (van Soest et al., 1991) and the crude protein estimated by multiplying N by 6.25. Ether extract was determined by the Soxhlet methodology while *in vitro* digestibility and metabolisable energy was estimated using near infrared reflectance spectroscopy (Garnsworthy and, Unal, 2004).

**Table 3.1** Chemical composition of feed components [1]

| | Feed components | | | Unit |
|---|---|---|---|---|
| | ***Spirulina*** | **Barley grain** | **Ryegrass pasture** | |
| Moisture[2] | 4.0 | 6.8 | 55.3 | g/100g Fresh Wt |
| DM | 96.0 | 93.2 | 44.7 | g/100g Fresh Wt |
| NDF | 32.6 | 18.5 | 22.4 | %DM |
| NDFn[3] | 30.3 | 17.2 | 20.8 | %DM |
| ADF | 18.3 | 6.0 | 23.0 | %DM |
| NFC[4] | 7.9 | 68.7 | 43.5 | %DM |
| Ash | 9.5 | 3.2 | 11.9 | %DM |
| EE | 5.9 | 2.0 | 3.0 | %DM |
| CP[5] | 61.0 | 11.5 | 20.8 | %DM |
| ME[6] | 1707.5 | 1723.7 | 1701.1 | kJ/100g DM |

[1] Dry matter (DM), neutral detergent fibre (NDF), nitrogen free NDF (NDFn), non fibrous carbohydrate (NFC), acid detergent fibre (ADF), ether extract (EE), indigestible organic matter (IOM), and metabolisable energy (ME)
[2] Moisture = 100 – DM
[3] NDFn = NDF x 0.93 (Undersander and, Moore, 2002)
[4] NFC = 100 – (NDFn + CP + EE + Ash) (Undersander and, Moore, 2002)
[5] CP = N x 6.25
[6] ME = 4194 – (9.2 x Ash) + (1.9 x CP) + (3.9 x EE) – (3.5 x NDF) [converted from kcal/kg] (Noblet and, Perez, 1993)

*Statistical analysis*

All data were analysed using the 'Statistical Analysis System' software package (SAS Institute., 2009). Initially, summary statistics by *Spirulina* supplementation level, sire breed and sex, were computed with means, standard deviations, and minimum and maximum values scrutinised for any data entry errors or outliers. Subsequently, factorial analysis of variance in generalised linear model (PROC GLM) and Mixed Model (PROC MIXED) analyses (SAS Institute., 2009) were used to fit the fixed effects of *Spirulina* supplementation level, sire breed, sex and their second-order interactions on wool FD, CV, SD, SF, CF, CURV, and YIELD. Sire was fitted as a random effect in the Mixed Model. Significant differences and mean separations were carried out using Duncan's multiple range and Bonferroni's probability pairwise comparison tests (SAS Institute., 2009). Pearson correlation coefficients between

dependent variables were estimated using PROC CORR (SAS Institute., 2009) with significance determined using Bonferroni's probability pairwise comparison test (SAS Institute., 2009).

**Results**

*Effect of* Spirulina *supplementation level, sire breed and sex on wool traits*

*Spirulina* supplementation level had no significant effect on any wool quality trait, compared to the CONTROL group ($P$>0.05; Table 3.2). However, wethers produced wool with lower FD ($P$<0.046), SD ($P$<0.046) and SF ($P$<0.019) than ewes. CF was lower in ewes than wethers (79.9 ± 3.31 and 88.1 ± 2.2%, respectively, Table 3.3).

Merino-sired lambs had lower FD (18.0 ± 0.1 µm), SF (17.1 ± 1.0 µm), CURV (63.5 ± 1.5 °/mm) and higher CF (96.2 ± 3.5 %) compared to all other sire breeds studied ($P$<0.001). Among the crossbreds, Black Suffolk-sired lambs had the highest SF (26.1 ± 0.6 µm) and Dorset-sired lambs the least (23.8 ± 0.9 µm; Table 3.3).

**Table 3.2** *Spirulina* supplementation level effect on physical wool traits least square means and standard errors, and level of significance ($P$ values) [1, 2]

| | *Spirulina* supplementation level | | | *P* values |
|---|---|---|---|---|
| | **CONTROL** | **LOW** | **HIGH** | |
| FD (µm) | 23.7 ± 1.1 | 24.6 ± 1.3 | 24.3 ± 1.2 | 0.687 |
| SD | 4.6 ± 0.3 | 4.4 ± 0.2 | 16.8 ± 1.2 | 0.542 |
| CV (%) | 19.5 ± 0.8 | 17.8 ± 0.5 | 5.1 ± 0.8 | 0.123 |
| CF (%) | 85.5 ± 3.0 | 81.8 ± 4.3 | 84.7 ± 3.5 | 0.620 |
| SF (µm) | 22.9 ± 1.0 | 23.4 ± 1.2 | 23.0 ± 1.1 | 0.865 |
| CURV (°/mm) | 71.4 ± 2.8 | 71.4 ± 2.3 | 73.8 ± 2.3 | 0.657 |
| YIELD (%) | 75.0 ± 1.4 | 72.7 ± 1.0 | 74.6 ± 1.0 | 0.173 |

[1] Row means bearing different superscripts significantly differ ($P$<0.05).
[2] Mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), and clean fleece yield (YIELD).

**Table 3.3** Sire breed and sex effect on physical wool traits least square means and standard errors, and level of significance (*P* values) [1, 2]

| | Wool characteristics | | | | | | |
|---|---|---|---|---|---|---|---|
| | FD (μm) | CV (%) | SD | CF (%) | SF (μm) | CURV (°/mm) | YIELD (%) |
| **Sire breed** | | | | | | | |
| Black Suffolk | 27.2 ± 0.7$^{A}$ | 19.4 ± 0.9 | 5.3 ± 0.2 | 74.5 ± 4.3$^{C}$ | 26.1 ± 0.6$^{A}$ | 74.3 ± 3.0$^{A}$ | 74.2 ± 1.3 |
| Dorset | 25.0 ± 0.9$^{A}$ | 18.1 ± 0.7 | 4.6 ± 0.3 | 85.4 ± 3.8$^{B}$ | 23.8 ± 0.9$^{B}$ | 75.2 ± 2.7$^{A}$ | 74.1 ± 1.2 |
| Merino | 18.0 ± 1.1$^{B}$ | 17.0 ± 1.4 | 4.4 ± 1.1 | 96.2 ± 3.5$^{A}$ | 17.1 ± 1.0$^{C}$ | 63.5 ± 1.5$^{B}$ | 75.2 ± 1.7 |
| White Suffolk | 26.7 ± 0.4$^{A}$ | 17.7 ± 1.1 | 4.7 ± 0.2 | 79.9 ± 2.1$^{BC}$ | 25.3 ± 0.3$^{AB}$ | 75.9 ± 2.5$^{A}$ | 72.7 ± 1.0 |
| *P* values | <0.001 | 0.474 | 0.703 | <0.001 | <0.001 | 0.004 | 0.399 |
| **Sex** | | | | | | | |
| Ewes | 25.1 ± 0.9$^{A}$ | 18.3 ± 1.0 | 5.3 ± 0.5$^{A}$ | 79.9 ± 3.3$^{B}$ | 24.0 ± 0.9$^{A}$ | 71.8 ± 1.8 | 74.8 ± 0.8 |
| Wethers | 23.4 ± 0.9$^{B}$ | 17.8 ± 0.4 | 4.1 ± 0.2$^{B}$ | 88.1 ± 2.2$^{A}$ | 22.2 ± 0.9$^{B}$ | 72.7 ± 2.2 | 73.4 ± 1.0 |
| *P* values | 0.046 | 0.620 | 0.046 | 0.016 | 0.019 | 0.721 | 0.186 |

[1] Column means within fixed effects bearing different superscripts significantly differ (*P*<0.05).
[2] Mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), and clean fleece yield (YIELD).

*Effect of interactions between Spirulina supplementation level and sex on wool traits*

It was evident from Figure 3.1 that ewes receiving HIGH *Spirulina* supplementation levels had higher wool YIELD than their wether counterparts (77.2 ± 0.8 % and 71.9 ± 1.4 % respectively). All other interactions between *Spirulina* supplementation level and sex were not significant (*P*>0.05; Supplementary Articles).

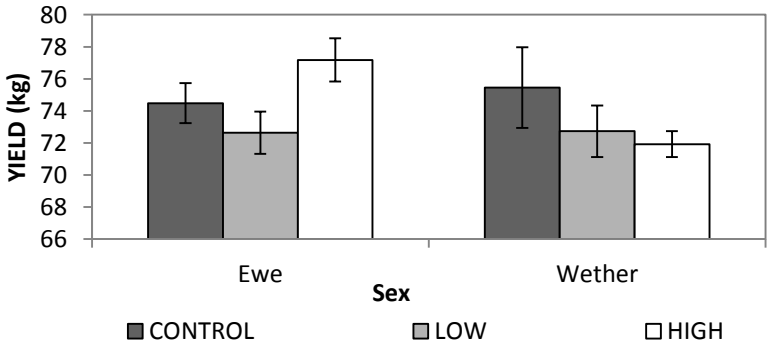


**Figure 3.1** *Spirulina* supplementation level and sex interactions on clean fleece percentage (YIELD; *P*<0.045)

*Effect of sire breed and sex interactions on wool traits*

Black Suffolk-sired ewe lambs had lower CURV than their wether counterparts, 67.8 ± 2.0 °/mm and 80.8 ± 3.0 °/mm respectively ($P$<0.032) as depicted in Figure 3.2. No other second-order interactions between sire breed and sex reached statistical significance ($P$>0.05).


**Figure 3.2** Sire breed and sex interactions on fibre curvature (CURV; $P$<0.032)

*Correlations between wool traits*

Table 3.4 shows that there were highly significant relationships ($P$<0.001) between FD and SF (0.99), CF with both FD (-0.87) and SF (-0.88), FD and CURV (0.39), SF and CURV (0.37), and SD with YIELD (0.29). All other correlations were not significant ($P$>0.05).

**Table 3.4** Physical wool traits Pearson's correlation coefficients [1, 2]

| | CV (%) | SD | CF (%) | SF (µm) | CURV (°/mm) | YIELD (%) |
|---|---|---|---|---|---|---|
| FD (µm) | $0.12^{NS}$ | $0.17^{NS}$ | -0.87*** | 0.99*** | 0.39** | $0.13^{NS}$ |
| CV | | $-0.27^{NS}$ | $-0.18^{NS}$ | $0.12^{NS}$ | $0.37^{NS}$ | $0.06^{NS}$ |
| SD | | | $-0.25^{NS}$ | $0.21^{NS}$ | $-0.07^{NS}$ | 0.29* |
| CF | | | | -0.88*** | $-0.87^{NS}$ | $0.04^{NS}$ |
| SF | | | | | 0.35* | $0.11^{NS}$ |
| CURV | | | | | | $0.21^{NS}$ |

[1] Level of significant: $^{NS}$ no significance ($P$>0.05), * significant ($P$<0.05), ** highly significant ($P$<0.01), *** very highly significant ($P$<0.001)

[2] Mean fibre diameter (FD), coefficient of variation (CV), standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), and clean fleece yield (YIELD).

**Discussion**

Wool growth and quality depend on the type of protein supplement, its nutritional value, and level of supplementation (Malau-Aduli et al., 2009e; Masters *et al.*, 1998; Rowe et al., 1989). Protein-rich supplements vary in amino acid availability thus affecting follicular uptake and wool fibre proliferation (Li et al., 2008). Therefore, increasing amino acid availability within the body-pool by increasing protein-rich supplementation, generally results in heightened follicular uptake that favours nutrient partitioning towards faster growth but coarser wool fibre synthesis in crossbred lambs (Malau-Aduli and, Holman, 2010; Malau-Aduli *et al.*, 2009c). This coarser fibre is characteristic of lesser quality wool (Holman and, Malau-Aduli, 2012a). However, in this study, there were no observable detrimental effects of *Spirulina* supplementation on wool quality traits.

*Spirulina* has a lower content of sulphur-containing amino acids compared to other protein-rich lamb supplements (Ciferri and, Tiboni, 1985; Volkmann et al., 2008). Methionine and cysteine are sulphur amino acids which are essential for wool fibre proliferation (Liu and, Masters, 2003). Cysteine plays a vital role during the differentiation of intermediate filament- and keratin associated proteins during wool synthesis (Plowman, 2007). Furthermore, methionine acts as a source of cysteine in the transulphuration pathway (Liu and, Masters, 2003). *Spirulina's* relatively minor content of these sulphur amino acids could likely explain insignificant differences in wool traits between the unsupplemented CONTROL and *Spirulina* supplemented group of lambs.

Ewes generally have lesser liveweights than wethers (Cam et al., 2010; Holman et al., 2012). This difference could result in considerable variation with other prioritised sinks requiring more of the available amino acids (Rogers and, Schlink, 2010). Our sex and sire breed findings were similar to published literature that have demonstrated hormonal differences between sexes (Egan and, Russell, 1981; Wallace, 1979) and wool follicle trait variations between sire breeds (Lee and, Williams, 1993; Scales et al., 2000) as having major impacts on wool traits. Similarly, the correlations between

wool traits is in line with published literature (Notter *et al.*, 2007). The strongly positive correlation between FD and SF is expected as SF is calculated from FD and CV values (Butler and, Dolling, 1992; Holman and, Malau-Aduli, 2012a). Likewise, CF represents the proportion of fibres over 30 µm (Holman and, Malau-Aduli, 2012a; Wood, 2003) and is a function of FD, thus the strong correlation between CF and FD. However, SF and CF had an antagonistic relationship, hence the negative correlation. The insignificant correlation between CF and YIELD with other wool traits has been previously reported (Hatcher et al., 2010).

**Conclusion**

In conclusion, the hypothesis that *Spirulina* supplementation via oral drenching will not be detrimental to wool quality in grazing purebred and crossbred Merino lambs holds true and should be accepted. The responses of lambs of different sire breeds and sex to *Spirulina* supplementation in terms of interaction effects on wool traits add to present knowledge. Finally, there is the need for further investigation into the underlying mechanisms behind our findings, particularly with regard to circulating plasma metabolites and proteomic profiles of supplemented lambs. This would provide a comprehensive understanding of *Spirulina*'s future applications as a protein-rich lamb feed supplement in Australian dual-purpose lamb operations.

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Chapter 4

# Effect of *Spirulina* supplementation level and basal diet on liveweight, body conformation and growth traits in dual-purpose lambs during simulated-drought and typical pasture grazing

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**ABSTRACT**

This study tested the effects of *Spirulina* supplementation level, basal diet and their interactions with sire breed and sex on liveweight, body conformation and growth traits in dual-purpose Australian lambs. In two concurrent feeding trials utilising typical pasture-based and simulated-drought basal diets, a total of 48 lambs was randomly allocated to treatment groups of *Spirulina* supplementation levels (CONTROL – 0ml, LOW – 50ml (only used in 1 trial), MEDIUM – 100ml and HIGH – 200ml), balanced by sire breed (Black Suffolk, Dorset, Merino and White Suffolk) and sex (ewes and wethers). In both feeding trials, *Spirulina* was supplemented daily for 9-weeks, following a 3-week adjustment phase. Weekly data of liveweight and body conformation measurements; chest girth (CG), wither height, body length, and body condition score (BCS), were taken throughout the trials. These were transformed into changes between initial and final periods of the feeding trial. Average daily liveweight gain (ADG) was computed from the differences in liveweight divided by duration in days. All data were analysed using Factorial ANOVA analysis in SAS. MEDIUM and HIGH *Spirulina* supplementation levels were found to improve the liveweight and ADG of White Suffolk- and Merino-sired lambs on simulated-drought basal diets. BCS improved with *Spirulina* supplementation in lambs on simulated-

drought basal diets. Under typical pasture-based basal diet, supplemented lambs had comparatively higher CG, BCS, liveweight and ADG than their counterparts on simulated-drought basal diets. These findings are of practical significance to the sheep industry because of the potential selection aid to Australian farmers in identifying the usefulness of *Spirulina* as a supplement and the optimal sire breed choice for the best performance response that is cost-effective when using dietary protein-rich supplements for their dual-purpose prime lamb operations.

(**Keywords**: *Sheep, protein supplementation, weight, crossbreds, nutritional plane*)

**Introduction**

*Spirulina* (*Arthrospira platensis*) is an edible cyanobacterium and has been the focus of a recent review of published research regarding its usefulness as a supplement in livestock feeds (Belay et al., 1996; Holman and, Malau-Aduli, 2012b). *Spirulina* has a complete nutrient and protein-rich constitution as depicted in Table 4.1. Yet, to the best of our current knowledge, *Spirulina*'s impact on growth in Australia's dual-purpose prime lamb industry during drought or typical pasture grazing periods remains unknown.

Crossbreeding and protein-rich supplementation are characteristic features of the Australian dual-purpose sheep industry that enhance lamb growth rates, the attainment of heavier liveweights (Hegarty et al., 2006a; Malau-Aduli *et al.*, 2009a), and premium meat prices (Martin, 2012). Generally, cereals and oil grains are used as supplements (Martin, 2012). However, the future availability of these traditional supplements has been questioned (Hegarty, 2012; Poppi and, McLennan, 2010). Consequently, alternative protein-rich supplements, such as *Spirulina*, must be investigated. Furthermore, any alternative supplement investigation must account for interactions with lamb sire breeds and basal diets which are typical of Australian sheep production.

**Table 4.5** Chemical composition of feed components

| | Feed components | | | | *Unit* |
|---|---|---|---|---|---|
| | *Spirulina* | Barley grain | Lucerne hay | Ryegrass pasture | |
| Moisture[2] | 4.0 | 6.8 | 9.4 | 55.3 | g/100g Fresh Wt |
| DM | 96.0 | 93.2 | 90.6 | 44.7 | g/100g Fresh Wt |
| NDF | 32.6 | 18.5 | 36.0 | 22.4 | %DM |
| NDFn[3] | 30.3 | 17.2 | 33.5 | 20.8 | %DM |
| ADF | 18.3 | 6.0 | 29.0 | 23.0 | %DM |
| NFC[4] | 7.9 | 68.7 | 35.2 | 43.5 | %DM |
| Ash | 9.5 | 3.2 | 6.9 | 11.9 | %DM |
| EE | 5.9 | 2.0 | 1.9 | 3.0 | %DM |
| CP[5] | 61.0 | 11.5 | 9.9 | 20.8 | %DM |
| ME[6] | 1707.5 | 1723.7 | 1689.3 | 1701.1 | kJ/100g DM |

[1] Dry matter (DM), neutral detergent fibre (NDF), nitrogen free NDF (NDFn), non fibrous carbohydrate (NFC), acid detergent fibre (ADF), ether extract (EE), indigestible organic matter (IOM), and metabolisable energy (ME)
[2] Moisture = 100 – DM
[3] NDFn = NDF x 0.93 (Undersander and, Moore, 2002)
[4] NFC = 100 – (NDFn + CP + EE + Ash) (Undersander and, Moore, 2002)
[5] CP = N x 6.25
[6] ME = 4194 – (9.2 x Ash) + (1.9 x CP) + (3.9 x EE) – (3.5 x NDF) [converted from kcal/kg] (Noblet and, Perez, 1993)

Liveweight is measured using sheep scales, and monitoring body conformation provides an alternative objective assessment of lamb growth in areas directly related to carcass yield and high value meat cuts (Cam et al., 2010). Use of body conformation measurements in conjunction with lamb liveweights allows individual lamb response to new feed supplements to be comprehensively quantified. Published literature on the growth and body conformation responses of prime lambs to *Spirulina* supplementation under typical pasture grazing and simulated drought conditions is currently non-existent and represents a major knowledge gap.

Therefore, this study aimed at testing the effect of *Spirulina* supplementation and its interactions with basal diet, sire breed and sex on crossbred and purebred Merino lambs' liveweights, body conformation and growth. It was hypothesised that lamb liveweight, body conformation measurements and growth will increase with an increase in the level of *Spirulina* supplementation, particularly under simulated drought conditions than during typical pasture grazing with significant interactions with sire breed and sex of lambs.

**Materials and methods**

This experiment was carried out at the University of Tasmania (UTAS) Farm, Cambridge, Hobart, Tasmania, Australia, in accordance with the 1993 Tasmanian Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004) and approved by the UTAS Animal Ethics Committee.

*Animal management and experimental design*

Over two consecutive years (2011-2012), a mating ratio of 1 terminal ram to 100 Merino ewes was used to produce approximately 1600 $F_1$ crossbred progeny. At 12 weeks of age, all progeny were identified using National Livestock Identification ear tags and weaned onto ryegrass pastures. At 6 months old, a total of 48 lambs was randomly selected for each feeding trial; 24 lambs on typical ryegrass pasture basal diet (Year 1); and, 24 lambs on simulated-drought basal diet of lucerne hay (Year 2). Each feeding trial continued for 9-weeks after three weeks of adjustment, during which commercially purchased *Spirulina* powder (TAAU, Darwin, Northern Territory, AUS) was directly supplemented to lambs by oral drenching daily in a water suspension at a *Spirulina* (g) : Water (mL) ratio of 1:10 w/v.

*Typical basal diet*: Random lamb allocation into the following treatment groups was undertaken: CONTROL (0mL), MEDIUM (100mL) and HIGH (200mL) *Spirulina* supplementation levels balanced by lamb sire breed (Black Suffolk, Dorset, Merino and White Suffolk) and sex (ewes and wethers). All lambs had *ad libitum* access to drinking water, 150g of barley grains per day and were run as a single flock on ryegrass pasture.

*Simulated-drought basal diet*: Treatment groups of *Spirulina* supplementation levels were: CONTROL (0mL), LOW (50mL), MEDIUM (100mL), and HIGH (200mL); sire breeds – Dorset, Merino and White Suffolk; and sex – ewes and wethers. Lambs were confined in individual 0.6m x 1.2m metabolic crates with *ad libitum* access to

drinking water and Lucerne hay, which was replaced daily. All lambs received barley (150g/day).

*Liveweight and body conformation measurements*

The following weekly body conformation measurements were taken using a measuring tape: chest girth (CG) as body circumference just behind the lamb's forelegs (Afolayan et al., 2006); wither height (WH) as the distance between the highest peak over the scapulae and the ground (Sowande and, Sobola, 2008); and body length (BL) as the span between the base of the neck and the far point of the pubic bone (Sowande and, Sobola, 2008). Body condition scores (BCS) were also subjectively assessed at weekly intervals throughout the trial by the same researcher to minimise variation, using a 0-5 point fat depth gauging scale (McLeod et al., 2010). Liveweights (BWT) were taken weekly using a calibrated Ruddweigh 3000XT walk-over weighing electronic scale with capability of automatic scanning of lamb identity and downloading of weight data directly into excel spreadsheet. All measurements were made while lambs were restrained and in a relaxed state, with heads comfortably erect and standing stably upon all four legs on flat ground to minimise stress.

*Data and statistical analysis*

Liveweight, BCS and body conformation measurements were transformed into changes over the duration of the feeding trial (*final minus initial values*). Average daily liveweight gain (ADG) was computed as liveweight change over the duration of the feeding trial divided by in the number of days.

All data were analysed using 'Statistical Analysis System' software (SAS Institute., 2009). Initial descriptive summary statistics were computed with means, standard deviations, and minimum and maximum values scrutinised for data entry errors and outliers. The data were then subjected to Factorial ANOVA (PROC GLM) analysis, with *Spirulina* supplementation level, sire breed, sex, basal diet and their

interactions fitted as fixed effects and BWT, CG, WH, BL, BCS and ADG as dependent variables. Level of significance threshold was $P<0.05$ and differences between means were established using Duncan's multiple range and Bonferroni's probability pairwise comparison tests.

**Results**

Variation in *Spirulina* supplementation levels resulted in significant differences in BWT ($P<0.049$) and ADG ($P<0.039$) with lambs in the LOW treatment group recording the lowest liveweight and daily gain compared to all other treatments (Table 4.2). Lambs receiving MEDIUM *Spirulina* supplementation gained and grew the most; a total BWT of 4.7 kg equivalent to an ADG of 76.6 g/day. Sex and type of basal diet were also significant sources of variation in lamb growth and body conformation traits as ewe lambs had higher CG (5.88 cm) than wether lambs (3.88 cm) and CG, BCS, BWT and ADG were all higher in lambs on a basal diet of typical ryegrass pastures than their counterparts on simulated-drought basal diet of lucerne hay (Table 4.2). All other independent fixed effects had no significant impact on liveweight, body conformation and growth ($P>0.05$; Table 4.2).

**Table 4.6** *Spirulina* supplementation level, sire breed, sex and plane of nutrition independent affects on change in liveweight, body conformation and growth least significant mean and standard error (LSM ± SEM), and level of significance (*P* values), over the feeding trial [1, 2]

| | Body conformation traits | | | | | |
|---|---|---|---|---|---|---|
| | **ΔCG (cm)** | **ΔWH (cm)** | **ΔBL (cm)** | **ΔBCS (0-5)** | **ΔBWT (kg)** | **ADG (g)** |
| ***Spirulina* level** | | | | | | |
| CONTROL | 5.07 ± 1.08 | 4.07 ± 0.46 | 3.79 ± 0.61 | 0.43 ± 0.16 | 4.29 ± 0.54$^{A}$ | 70.61 ± 9.19$^{A}$ |
| LOW | 2.33 ± 0.92 | 4.83 ± 0.60 | 5.33 ± 0.71 | 0.50 ± 0.18 | 2.50 ± 0.22$^{B}$ | 39.68 ± 3.55$^{B}$ |
| MEDIUM | 4.93 ± 0.59 | 3.93 ± 0.43 | 4.29 ± 0.55 | 0.54 ± 0.11 | 4.68 ± 0.35$^{A}$ | 76.59 ± 5.94$^{A}$ |
| HIGH | 5.21 ± 0.83 | 3.71 ± 0.44 | 4.43 ± 0.27 | 0.50 ± 0.10 | 3.89 ± 0.75$^{A}$ | 64.12 ± 12.36$^{A}$ |
| *P values* | *0.711* | *0.440* | *0.755* | *0.658* | *0.049* | *0.039* |
| **Sire breed** | | | | | | |
| Black Suffolk | 4.83 ± 0.40 | 4.00 ± 0.68 | 3.17 ± 0.48 | 0.83 ± 0.17 | 4.25 ± 0.42 | 70.83 ± 7.05 |
| Dorset | 5.21 ± 0.62 | 3.50 ± 0.40 | 4.50 ± 0.58 | 0.57 ± 0.10 | 3.75 ± 0.77 | 61.42 ± 12.69 |
| Merino | 5.07 ± 1.20 | 4.50 ± 0.43 | 4.57 ± 0.44 | 0.21 ± 0.13 | 4.14 ± 0.50 | 67.69 ± 8.42 |
| White Suffolk | 3.86 ± 0.80 | 4.07 ± 0.46 | 4.36 ± 0.52 | 0.54 ± 0.12 | 4.21 ± 0.47 | 68.85 ± 8.03 |
| *P values* | *0.388* | *0.467* | *0.898* | *0.081* | *0.294* | *0.272* |
| **Sex** | | | | | | |
| Ewe | 5.58 ± 0.72$^{A}$ | 4.00 ± 0.30 | 4.38 ± 0.36 | 0.46 ± 0.08 | 3.92 ± 0.50 | 64.35 ± 8.23 |
| Wether | 3.88 ± 0.51$^{B}$ | 4.04 ± 0.36 | 4.25 ± 0.41 | 0.52 ± 0.11 | 4.21 ± 0.34 | 68.83 ± 5.77 |
| *P values* | *0.046* | *0.931* | *0.816* | *0.621* | *0.550* | *0.569* |
| **Basal diet** | | | | | | |
| Typical pasture | 5.96 ± 0.65$^{A}$ | 4.13 ± 0.35 | 3.63 ± 0.23 | 0.65 ± 0.08$^{A}$ | 5.31 ± 0.27$^{A}$ | 88.54 ± 4.56$^{A}$ |
| Simulated-drought | 3.50 ± 0.55$^{B}$ | 3.92 ± 0.32 | 5.00 ± 0.45 | 0.33 ± 0.10$^{B}$ | 2.81 ± 0.39$^{B}$ | 44.64 ± 6.23$^{B}$ |
| *P values* | *0.015* | *0.322* | *0.079* | *0.041* | *<0.001* | *<0.001* |

[1] Column means within a fixed effect bearing different superscripts differ (P<0.05).
[2] Chest girth (CG), withers height (WH), body length (BL), body condition score (BCS), body weight (BWT), and average daily weight gain (ADG).

Supplementary tables portray the second and third order interaction effects of *Spirulina* supplementation level, basal diet, sire breed and sex on changes in lamb liveweight, body conformation and growth. Some of the most significant interactions are depicted in Figures 4.1-4.3. It was apparent that lambs on simulated drought basal diets and supplemented with *Spirulina* at all levels had higher BCS and CG than their unsupplemented counterparts in the control treatment group and the highest BWT and ADG responses were obtained at MEDIUM levels of supplementation (Figure 4.1).

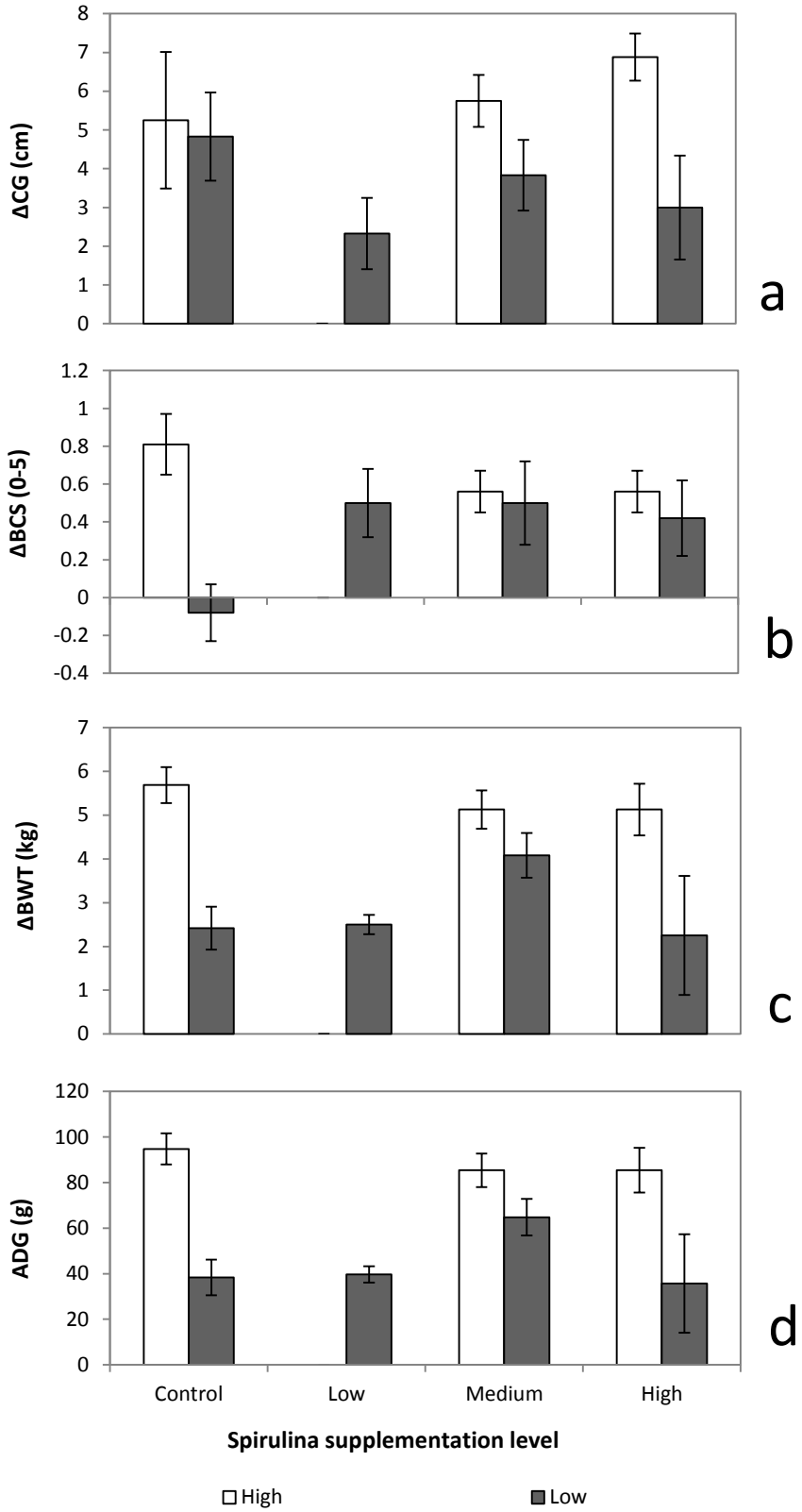


**Figure 4.3** *Spirulina* supplementation level interactions with plane of nutrition on change in: a) chest girth (ΔCG; *P*<0.039); b) body condition score (ΔBCS; *P*<0.018); c) liveweight (ΔBWT; *P*<0.001); and d) average daily gain (ADG; *P*<0.001). *Note: High plane of nutrition did not include Low Spirulina supplementation level.*

In terms of sire breed and supplementation level interactions, Figure 4.2 shows that purebred Merino and White Suffolk-sired lambs at MEDIUM and HIGH levels of supplementation had higher BWT and ADG than unsupplemented lambs in the control group. However, Merino-sired lambs on simulated-drought basal diets had lower BCS compared with their peers on typical pasture-based basal diets (Figure 4.3).

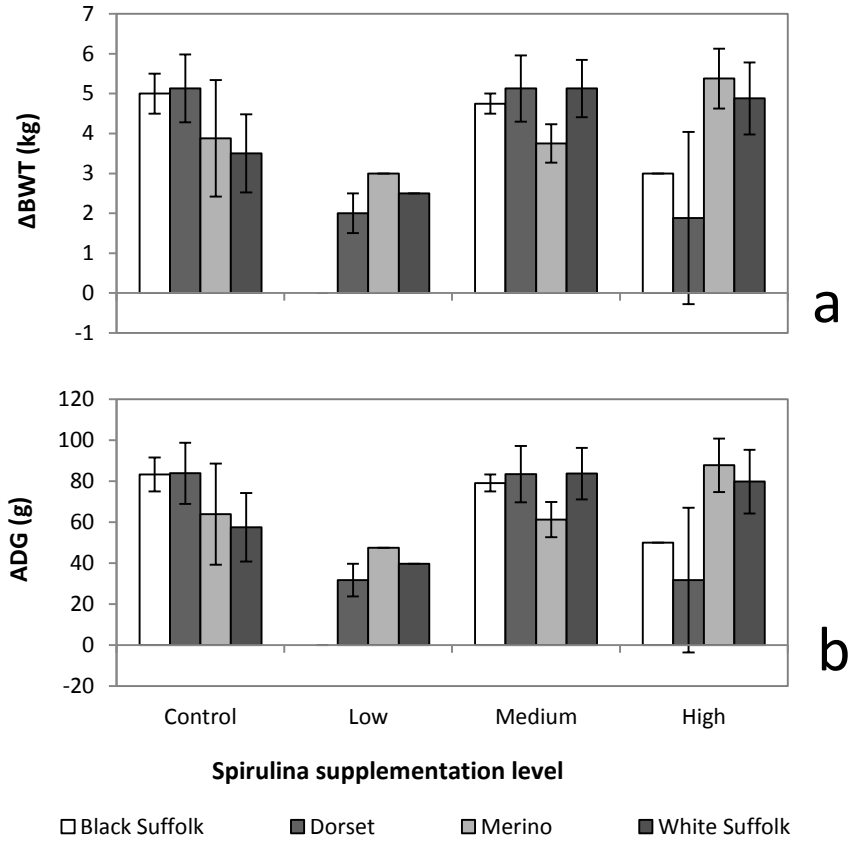


**Figure 4.4** *Spirulina* supplementation level and sire breed interactions on change in: a) liveweight (ΔBWT; *P*<0.041); and b) average daily gain (ADG; *P*<0.039).

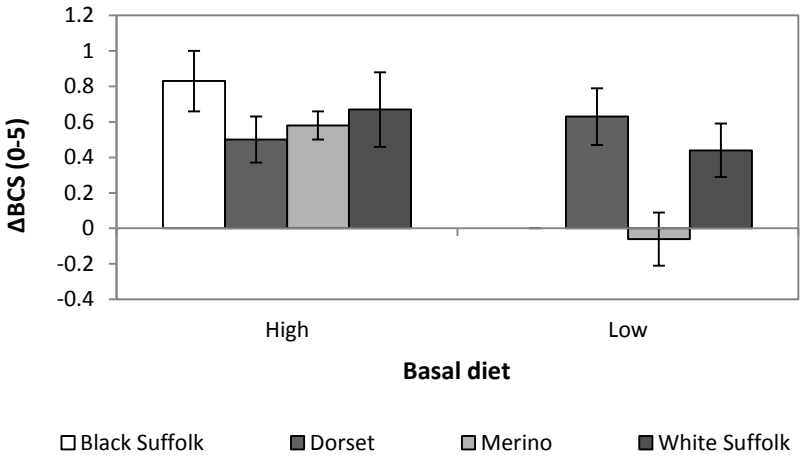


**Figure 4.5** Sire breed and plane of nutrition interactions on change in body condition score (ΔBCS; *P*<0.025). *Note: Low basal diet did not include Black Suffolk sire breed*.

**Discussion**

This research tested the hypothesis that lamb liveweight, body conformation measurements and growth will increase with an increase in the level of *Spirulina* supplementation, particularly under simulated drought conditions than during typical pasture grazing with significant interactions with sire breed and sex of lambs. This study has established that there are beneficial effects of supplementing dual-purpose prime lambs with *Spirulina*, particularly during drought periods, and our findings are in congruence with other protein-rich supplements in previous studies (Hussein and, Jordan, 1991; Karlsson and, Martinsson, 2011).

Increased dietary protein, through supplementation, contributes to meeting the requirements of muscle mass accretion in lambs (NRC., 1985). However, once these protein requirements are met, excess protein is generally deaminated and excreted in urine without further impact on lamb liveweights or growth rates. This effect was implied in this study as MEDIUM and HIGH supplementation levels did not differ in lamb body conformation and growth responses. Other studies have reported similar conclusions (Bezerra et al., 2010; Holman et al., 2012). Malau-Aduli *et al*. (2009b) reported that crossbred lambs provided intermediate levels of protein-rich

supplements outperformed their higher supplemented counterparts. These suggest that dual-purpose lamb producers are economically rewarded by supplementing lambs at medium levels that are not only efficient, but also cheaper than high levels of supplementation.

The observed divergences in lamb responses to *Spirulina* supplementation with basal diet could have arisen from differences in feed intake levels as pasture was more nutritious than the simulated-drought basal diet of lucerne hay (Table 4.1).

Lewis *et al.* (2006) demonstrated that lambs from pedigrees with high growth exceeded other lambs from low growth parental lineage in terms of liveweights and growth rates. Hence, the availability and partitioning of nutrients from consumed feeds and supplements are under genetic control (Cronjé and, Boomker, 2000). In this study, the differences in lamb response to basal dietary intake and *Spirulina* supplementation between sire breeds is a pointer to the impact of sheep genetics on feed use efficiency and lamb predisposition to high liveweights and growth as widely acknowledged in published literature (Hegarty et al., 2006a; Ponnampalam et al., 2007).

Lamb liveweight and growth are derivatives of feed intake and nutrition. Consequently, any difference in basal diet is expected to be reflected in liveweight, body conformation and growth differences between lambs as shown in this study. Previous studies support this fact with lambs fed at higher planes of nutrition having greater growth rates and liveweights compared to lambs fed at lower planes of nutrition (Lee, 1986; Murphy *et al.*, 1994; Pálsson and, Vergés, 1952). Drought basal diets have been shown not to induce 'miniaturisation', instead different anatomical regions and tissue deposits are affected (Pálsson and, Vergés, 1952). Consequently, body conformation measurements vary in there indication of lamb response to diet.

Independent effects of sire breed and sex on lamb liveweight, body conformation and growth have previously been demonstrated (Afolayan et al., 2006; Cam et al., 2010; Ponnampalam et al., 2007). However, this study focussed on the changes in

liveweight, body conformation and growth over the duration of the feeding trial. It was interesting that significant interactions between level of supplementation, sire breed, basal diet and sex were detected as these provide sheep farmers with a spectrum of choices and sire breed combinations for targeting optimal liveweights and the attainment of slaughter weights.

**Conclusions**

*Spirulina* supplementation improves Australian dual-purpose lamb liveweights. However, this improvement is dependent on lamb supplementation level, sire breed and type of basal diet. MEDIUM *Spirulina* supplementation levels offer the most economical means to improve liveweight and growth under drought conditions. Merino- and White Suffolk-sired lambs responded best to *Spirulina* supplementation. Typical pasture grazing basal diet resulted in lambs outperforming their counterparts on simulated-drought basal diets with regard to chest girth, body condition score, liveweight and growth. These findings support the hypothesis that lamb liveweight, body conformation measurements and growth will increase with an increase in the level of *Spirulina* supplementation, particularly under simulated drought conditions than during typical pasture grazing with significant interactions with sire breed and sex of lambs.

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Chapter 5

# Liveweight, body conformation and growth responses to *Spirulina* supplementation in prime lambs on typical pasture-based high plane of nutrition

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**ABSTRACT**

The hypothesis that supplementation with *Spirulina* will increase liveweight, growth and body conformation with significant interactions between sire breed and sex was tested using purebred Merino weaners and first-cross weaners from Merino dams sired by Dorset, Black Suffolk and White Suffolk rams under the same pasture-based management system. Our experimental objective was to evaluate the effects of varying levels of *Spirulina* supplementation, sire breed and gender on liveweight and body conformation traits. We utilised a 4 x 3 x 2 factorial experimental design in which 24 prime lambs with an average liveweight of 37.6 ± 5.2 kg and body condition score of 3.1 ± 0.4 at 6 weeks of age were balanced by sire breed and gender and randomly allocated into 3 treatments (8 lambs per treatment) – the control group grazing without *Spirulina* (0%), MEDIUM (10% wt/vol – 100ml) and HIGH (20% wt/vol – 200 ml) *Spirulina*. Lambs in the MEDIUM and HIGH *Spirulina* treatment groups were drenched daily with *Spirulina* solution prior to being released for grazing with the control group of lambs over a 6-week trial period, following a 3-week adjustment phase. Weekly measurements of chest girth, withers height, body length, body condition score and liveweight were taken. Mixed linear model procedures in SAS with sire breed, sex, *Spirulina* level and their second order interactions as fixed effects and sire as a random variable, were used for statistical analysis. *Spirulina*

level significantly influenced lamb liveweight (*P*<0.018), body condition score (*P*<0.001) and body length (*P*<0.015). Lambs on MEDIUM *Spirulina* levels (10%) recorded the highest mean liveweight of 41.9 ± 0.7 kg. HIGH *Spirulina* levels (20%) did not significantly improve liveweight compared to the control group (0%). Highly significant sire breed interactions with *Spirulina* level (*P*<0.001) resulted in the heaviest (47.08 kg) and lightest (35.14 kg) average liveweights in Black Suffolk-sired crossbreds and purebred Merino lambs respectively, supplemented at the HIGH *Spirulina* level. Body conformation (P<0.001) and liveweight (*P*<0.014) responses to *Spirulina* supplementation significantly varied between ewe and wether lambs. It was evident that a cost-effective supplementation strategy with *Spirulina* for optimal liveweight gains in weaner lambs was achieved at the MEDIUM level. These findings will aid sheep farmers in making informed choices about appropriate sire breed and gender combinations in their enterprises when supplementing with *Spirulina* for growth improvement as a strategic pathway for the early attainment of market weights in prime lambs. We concluded that based on the empirical experimental evidence within the scope of this study, the tested hypothesis is acceptable.

(**Keywords**: *Spirulina, Merino, growth, sire breed, liveweight, crossbred, body conformation*)

**Introduction**

*Spirulina* (*Arthrospira platensis*) is a blue-green cyanobacterial alga with an extensive history of human consumption whereas in animal feeds, it has been adopted in the last two decades (Belay et al., 1993; Gupta *et al.*, 2008). *Spirulina* is 60-70% protein by weight (Belay et al., 1993; Doreau et al., 2010) and contains high levels of carotenoids, essential vitamins, minerals and fatty acids (Kistanova *et al.*, 2009; Panjaitan *et al.*, 2010; Toyomizu et al., 2001). *Spirulina* can be cultivated in a liquid medium (Volkmann et al., 2008) and has been found to out-yield traditional protein-rich feed sources like soybeans, and grains such as wheat, corn and barley in terms of production per land unit (Dismukes et al., 2008; Kulpys et al., 2009). Subsequently, supplementation with *Spirulina* has been trialled in sheep, cattle, swine and poultry

(Bezerra et al., 2010; Panjaitan et al., 2010; Toyomizu et al., 2001). Although, most of these studies are still in their infancy, ruminants have so far been identified as well suited to *Spirulina* supplementation due to their capacity to digest unprocessed algal material (Gouveia et al., 2008). Furthermore, *Spirulina* supplementation has been associated with heightened rumen microbial crude protein production (Panjaitan et al., 2010).

There is currently an increasing demand for sheep meat, particularly prime lamb. This follows heightened domestic and export market demands from Australia's traditional export markets in the US and EU, and emerging Asian markets (Hopkins et al., 2007a; McLeod et al., 2010; Rowe, 2010). This trend is expected to continue into the foreseeable future (Martin and, Phillips, 2011). About 80% of Australia's national flock remains predominantly Merino-based, a wool specialist breed, regardless of the current relatively lower economic value of wool (Greeff et al., 2008; Hatcher and, Atkins, 2000a; Swan, 2010; Warner et al., 2007). Hence, to capitalise on the present high demand for prime lamb, farmers are using selective crossbreeding strategies wherein meat-type terminal sires are joined with purebred Merino ewes (Ingham et al., 2007; Kopke et al., 2008). This permits the exploitation of individual and maternal heterosis, and blends desirable meat and wool traits into a single 'dual-purpose' lamb (Fogarty et al., 2006; Rowe, 2010; Safari et al., 2005; Thornton, 2010).

Lamb productivity is a derivative of feed nutrition (Black, 1983; Geesink and, Zerby, 2010; Hopkins et al., 2007b; McLeod et al., 2010) as growth rates and liveweights are foremost indicators of operational profitability (Afolayan et al., 2006; Cam et al., 2010). Profitability in dual-purpose meat sheep production is driven primarily by protein-rich feed supplementation. However, viability in the production of traditional protein-rich feeds like canola and lupins is dependent on unpredictable variations in climate and land availability (Poppi and, McLennan, 2010; Smith et al., 2010). Therefore, feed-lotting (Gaunt *et al.*, 2010) and grain finishing (Martin and, Phillips, 2011) in prime lamb production are gaining increasing popularity, particularly in drought-prone regions. Consequently, the identification of alternative

protein-rich feed sources is imperative in dealing with volatile production costs and variable climatic conditions (Poppi and, McLennan, 2010).

Our experimental objective was to evaluate liveweight, growth and body conformation responses of genetically divergent weaner prime lambs to *Spirulina* supplementation under typical pasture-based basal diet and to estimate phenotypic correlations between growth and body conformation traits under the same management system.

**Materials and Methods**

*Animal welfare and ethics clearance*

This experiment was approved by the University of Tasmania Animal Ethics Committee, and was conducted from April to June 2011 at the University of Tasmania Farm, Cambridge, Hobart, Australia. All procedures had the University of Tasmania Animal Ethics approval and were conducted in accordance with the 1993 Tasmanian Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004).

*Experimental design and animal management*

Twenty-four weaned lambs from purebred Merino dams sired by Dorset, White Suffolk, Black Suffolk and Merino rams lambs with an average liveweight of 37.6 ± 5.2 kg and body condition score of 3.1 ± 0.4 at 6 weeks of age were balanced by sire breed and gender and randomly allocated into 3 treatments (8 lambs per treatment) – the control group grazing without *Spirulina* (0%), MEDIUM (10%wt/vol – 100ml) and HIGH (20%wt/vol – 200ml) *Spirulina* levels. A 4 x 3 x 2 factorial experimental design representing 4 sire breeds (Dorset, White Suffolk, Black Suffolk, Merino), 3 *Spirulina* treatment levels (0% - control, 10% - MEDIUM, 20% wt/vol - HIGH), and sex (ewes, wethers) was utilized. After an initial 3-week adjustment phase, the supplementation and grazing trial continued for 6-weeks. Lambs were daily

supplemented according to their assigned *Spirulina* treatment before being released into paddocks for grazing with the control group of lambs. The *Spirulina* was obtained from a commercial retailer in powdered form (TAAU, Australia) and its chemical composition was: moisture (5.0 g/100g), fat (4.1 g/100g), protein (62.0 g/100g), ash (11.4 g/100g) and carbohydrate (17.5 g/100g) (Lopez, 2004). Hence, the treatment was delivered as a 1 g : 10 ml solution in water using a sheep drenching gun. After receiving their daily *Spirulina* drench, all lambs were released into phalaris/fescue mixed pasture paddocks for grazing as per normal commercial sheep production and had *ad libitum* access to drinking water.

*Liveweight and body conformation measurements*

At weekly intervals, each lamb was individually assessed for chest girth (CG), withers height (WH), body length (BL), body condition score (BCS) and liveweight (BWT) measurements. CG was the body circumference measured at just behind the forelegs (Afolayan et al., 2006). WH was the distance between the highest peak over the scapulae and the ground (Sowande and, Sobola, 2008). BL refers to the span between the base of the neck, the vertebrae between the scapulae, to the far point of the pubic bone (Sowande and, Sobola, 2008). BCS was subjectively measured (Phythian et al., 2011), always by the same researcher, gauging fat depth on a 0-5 point scale as described by McLeod *et al.* (2010). BWT was monitored using an electronic walk-over weighing scale equipped with an automatic sheep ID scanning digitally downloadable data capability. Body conformation measurements in centimetres were taken using the same measuring tape. During assessment it was ensured that lambs were gently restrained in a relaxed state on all four legs with their heads comfortably erect.

*Statistical analysis*

Using BWT values, the average daily gain (ADG) of individual lambs was computed in kg/day by dividing the weight change by the interval between weighings. All data were analysed using 'Statistical Analysis System' software (SAS Institute., 2009).

Initially, summary statistics by sex, sire breed and *Spirulina* level were computed. The means, standard deviations, minima, maxima and range of values were examined for data entry errors or outliers. Factorial ANOVA (PROC GLM) and Mixed Model (PROC MIXED) (SAS Institute., 2009) procedures were used to fit the fixed effects of *Spirulina* level, sire breed, sex and their second-order interactions on BWT, ADG, CG, WH, BL, BCS. Sire was fitted as a random effect in the mixed model. Separation of mean differences using Duncan's multiple range tests and Bonferroni pairwise comparison tests was conducted at $P<0.05$ level of significance. Pearson's correlation coefficients (PROC CORR) between dependent variables were also estimated and significance established using Bonferroni tests (SAS Institute., 2009).

**Results**

*Effect of Spirulina level, sire breed and sex on growth and body conformation traits*

*Spirulina* supplementation caused lambs to grow longer bodies (BL) than the control group ($P<0.015$), although no significant differences were detected between 10% and 20% *Spirulina* levels (Table 5.1). Lambs in the 20% *Spirulina* treatment group had greater BCS (3.4 ± 0.1) than their counterparts in the 10% and 0% (control) treatment groups ($P<0.001$). It was also evident that lambs receiving 10% *Spirulina* levels recorded the heaviest BWT of 41.9 ± 0.7 kg ($P<0.018$), but there were no BWT differences between the 20% and control treatment groups. Moreover, *Spirulina* supplementation level did not affect CG ($P>0.376$), WH ($P>0.669$) or ADG ($P>0.759$).

All body measurements were influenced by sire breed ($P<0.0001$; Table 5.1). Black Suffolk-sired lambs had the largest CG (99.0 ± 0.7 cm) while Merino-sired lambs had the smallest average WH (61.6 ± 0.4 cm). Black Suffolk-sired lambs had the longest BL (67.0 ± 0.3 cm), while White Suffolk- and Dorset-sired lambs did not significantly differ, although had longer BL than Merino-sired lambs (62.6 ± 0.5 cm). Lamb BCS and BWT followed similar trends among sire breeds in which Black Suffolk-sired lambs had the highest BCS (3.7 ± 0.1) and BWT (46.3 ± 0.6 kg). White Suffolk- and Dorset-sired lambs BCS and BWT proved similar, but significantly higher than

Merino-sired lambs, whose average BCS (3.1 ± 0.03) and BWT (33.5 ± 0.4 kg) were the lowest. ADG did not differ regardless of sire breed (*P*>0.502).

CG, WH and BWT were all significantly affected by sex (Table 5.1). Wethers had larger CG (96.2 ± 0.5 cm) and WH (63.4 ± 0.3 cm) than ewes (94.9 ± 0.6 cm and 62.4 ± 0.3 cm respectively). Correspondingly, mean BWT was heavier in wethers (42.1 ± 0.5 kg) than ewes (40.1 ± 0.6 kg; *P*<0.001). BL (*P*>0.346), BCS (*P*>0.346) and ADG (*P*>0.605) did not differ between sexes (Table 5.1).

**Table 5.7** Liveweight, body conformation, condition score and average daily gains under typical plane of nutrition least square means and standard error (LSM ± SE) [1, 2]

| | Body conformation traits | | | | | |
|---|---|---|---|---|---|---|
| | **CG (cm)** | **WH (cm)** | **BL (cm)** | **BCS (1-5)** | **BWT (kg)** | **ADG (kg/day)** |
| ***Spirulina* level** | | | | | | |
| CONTROL | 95.0 ± 0.6 | 62.9 ± 0.4 | 65.7 ± 0.4$^{B}$ | 3.2 ± 0.1$^{B}$ | 40.6 ± 0.7$^{B}$ | 0.2 ± 0.0 |
| 10% (MEDIUM) | 95.6 ± 0.6 | 62.7 ± 0.4 | 66.6 ± 0.4$^{A}$ | 3.3 ± 0.0$^{B}$ | 41.9 ± 0.7$^{A}$ | 0.1 ± 0.0 |
| 20% (HIGH) | 96.1 ± 0.7 | 63.1 ± 0.3 | 66.8 ± 0.4$^{A}$ | 3.4 ± 0.1$^{A}$ | 40.8 ± 0.6$^{B}$ | 0.1 ± 0.0 |
| *P-values* | *0.376* | *0.669* | *0.015* | *<0.001* | *0.018* | *0.759* |
| **Sire breed** | | | | | | |
| Black Suffolk | 99.0 ± 0.7$^{A}$ | 63.6 ± 0.4$^{A}$ | 68.8 ± 0.3$^{A}$ | 3.7 ± 0.1$^{A}$ | 46.3 ± 0.6$^{A}$ | 0.1 ± 0.0 |
| Dorset | 93.8 ± 0.5$^{B}$ | 63.5 ± 0.4$^{A}$ | 66.9 ± 0.3$^{B}$ | 3.2 ± 0.0$^{B}$ | 41.8 ± 0.4$^{B}$ | 0.2 ± 0.0 |
| Merino | 95.0 ± 0.9$^{B}$ | 61.6 ± 0.4$^{B}$ | 62.6 ± 0.5$^{C}$ | 3.1 ± 0.0$^{C}$ | 33.5 ± 0.4$^{C}$ | 0.1 ± 0.0 |
| White Suffolk | 94.4 ± 0.7$^{B}$ | 62.8 ± 0.4$^{A}$ | 67.0 ± 0.4$^{B}$ | 3.3 ± 0.1$^{B}$ | 42.9 ± 0.5$^{B}$ | 0.2 ± 0.0 |
| *P-values* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *0.502* |
| **Sex** | | | | | | |
| Ewes | 94.9 ± 0.6$^{B}$ | 62.4 ± 0.3$^{B}$ | 66.2 ± 0.4 | 3.3 ± 0.0 | 40.1 ± 0.6$^{B}$ | 0.1 ± 0.0 |
| Wethers | 96.2 ± 0.5$^{A}$ | 63.4 ± 0.3$^{A}$ | 66.5 ± 0.3 | 3.3 ± 0.0 | 42.1 ± 0.5$^{A}$ | 0.1 ± 0.0 |
| *P-values* | *0.034* | *0.009* | *0.269* | *0.346* | *<0.001* | *0.605* |

[1] Column means within a fixed effect bearing different superscripts differ (P<0.05).
[2] Chest girth (CG), withers height (WH), body length (BL), body condition score (BCS), body weight (BWT), and average daily weight gain (ADG)

*Effect of sire breed and sex interactions*

Merino-sired wether lambs had larger CG of 98.2 cm than ewe lambs (91.9 cm; *P*<0.001; Table 5.2). BL of Black Suffolk-sired ewes was longer (69.4 cm) than that of wethers (68.2 cm; *P*<0.001). A similar trend was also observed in the BL of Merino-sired lambs (61.3 cm in ewes and 64.0 cm in wethers). Mean WH was higher in wethers than ewes in Black Suffolk- (64.6 cm and 62.7 cm, respectively) and White Suffolk-sired lambs (66.7 cm and 67.4 cm, respectively; *P*<0.001). White Suffolk-sired

wether lambs also had greater BCS (3.4) than ewe lambs (*P*<0.001). BWT in Merino-sired wethers (35.2 kg) was heavier than in their ewe counterparts (31.7 kg). A similar scenario was also observed in Black Suffolk-sired lambs (47.1 kg and 45.5 kg in wether and ewe lambs, respectively; *P*<0.014).

**Table 5.8** Sire breed and sex interactions on liveweight, body conformation and average daily gains under typical plane of nutrition mean values [1, 2]

| Sire breed | Sex | Body conformation traits | | | | | |
|---|---|---|---|---|---|---|---|
| | | **CG (cm)** | **WH (cm)** | **BL (cm)** | **BCS (1-5)** | **BWT (kg)** | **ADG (kg/day)** |
| Black Suffolk | Ewe | $98.6^{A}$ | $62.7^{BCD}$ | $69.4^{A}$ | $3.7^{A}$ | $45.5^{B}$ | 0.12 |
| | Wether | $99.5^{A}$ | $64.6^{AB}$ | $68.2^{BC}$ | $3.8^{A}$ | $47.1^{A}$ | 0.14 |
| Dorset | Ewe | $94.3^{BC}$ | $63.5^{ABC}$ | $66.5^{CD}$ | $3.3^{BCD}$ | $41.1^{DE}$ | 0.14 |
| | Wether | $93.4^{BCD}$ | $63.5^{ABC}$ | $67.2^{BCD}$ | $3.1^{CDE}$ | $42.5^{CDE}$ | 0.15 |
| Merino | Ewe | $91.9^{CD}$ | $61.3^{CD}$ | $61.3^{F}$ | $3.0^{DE}$ | $31.7^{G}$ | 0.11 |
| | Wether | $98.2^{A}$ | $61.9^{CD}$ | $64.0^{E}$ | $3.1^{CDE}$ | $35.2^{F}$ | 0.11 |
| White Suffolk | Ewe | $94.8^{BC}$ | $62.1^{CD}$ | $67.4^{BCD}$ | $3.2^{CDE}$ | $42.2^{CDE}$ | 0.15 |
| | Wether | $94.00^{BCD}$ | $63.5^{ABC}$ | $66.7^{CD}$ | $3.4^{BC}$ | $43.7^{CD}$ | 0.17 |
| *P values* | | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *0.014* | *0.977* |

[1] Column means within second-order interactions bearing different superscripts differ (P<0.05).
[2] Chest girth (CG), withers height (WH), body length (BL), body condition score (BCS), body weight (BWT), and average daily weight gain (ADG)

*Effect of sire breed and Spirulina supplementation level interactions*

Black Suffolk-sired lambs in the 20% *Spirulina* supplementation group had the largest CG (101.9 cm), WH (65.1 cm), BL (68.7 cm) and BWT (47.1 kg) (*P*<0.001; Table 5.3). ADG was not influenced by sire breed and *Spirulina* level interactions as there were no distinct and consistent patterns (*P*>0.937; Table 5.3)

**Table 5.9** *Spirulina* supplementation level and sire breed interactions on liveweight, body conformation and average daily gains under typical plane of nutrition mean values [1, 2]

| *Spirulina* | Sire breed | Body conformation traits | | | | | |
|---|---|---|---|---|---|---|---|
| | | **CG (cm)** | **WH (cm)** | **BL (cm)** | **BCS (1-5)** | **BWT (kg)** | **ADG (kg/day)** |
| Black Suffolk | CONTROL | 97.0$^{A}$ | 62.9$^{A}$ | 68.2$^{A}$ | 3.5$^{A}$ | 44.7$^{A}$ | 0.15 |
| | 10% (MEDIUM) | 98.3$^{A}$ | 62.9$^{A}$ | 69.6$^{A}$ | 3.6$^{A}$ | 47.0$^{B}$ | 0.16 |
| | 20% (HIGH) | 101.9$^{B}$ | 65.1$^{B}$ | 68.7$^{A}$ | 4.1$^{B}$ | 47.1$^{B}$ | 0.09 |
| Dorset | CONTROL | 93.2$^{A}$ | 63.5$^{AB}$ | 66.0$^{A}$ | 3.1$^{A}$ | 41.5$^{AB}$ | 0.17 |
| | 10% (MEDIUM) | 95.1$^{A}$ | 64.5$^{B}$ | 67.2$^{A}$ | 3.2$^{A}$ | 43.2$^{A}$ | 0.14 |
| | 20% (HIGH) | 93.2$^{A}$ | 62.6$^{A}$ | 67.4$^{A}$ | 3.3$^{A}$ | 40.8$^{B}$ | 0.13 |
| Merino | CONTROL | 94.6$^{AB}$ | 61.6$^{AB}$ | 60.6$^{A}$ | 3.0$^{A}$ | 32.1$^{A}$ | 0.12 |
| | 10% (MEDIUM) | 93.4$^{B}$ | 60.5$^{A}$ | 62.5$^{B}$ | 3.1$^{A}$ | 33.1$^{A}$ | 0.10 |
| | 20% (HIGH) | 97.1$^{A}$ | 62.7$^{B}$ | 64.8$^{C}$ | 3.1$^{A}$ | 35.1$^{B}$ | 0.12 |
| White Suffolk | CONTROL | 95.2$^{AB}$ | 63.5$^{A}$ | 67.9$^{A}$ | 3.3$^{A}$ | 44.2$^{A}$ | 0.16 |
| | 10% (MEDIUM) | 95.8$^{B}$ | 63.00$^{A}$ | 67.1$^{A}$ | 3.3$^{A}$ | 44.4$^{A}$ | 0.15 |
| | 20% (HIGH) | 92.1$^{A}$ | 62.1$^{A}$ | 66.1$^{B}$ | 3.2$^{A}$ | 40.2$^{B}$ | 0.17 |
| *P values* | | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *0.936* |

[1] Column means within sire breed bearing different superscripts differ ($P$<0.05).
[2] Chest girth (CG), withers height (WH), body length (BL), body condition score (BCS), body weight (BWT), and average daily weight gain (ADG)

*Effect of sex and Spirulina supplementation level interactions*

*Spirulina* level and sex interactions were found to impact lamb CG ($P$<0.001), BL ($P$<0.001), BWT ($P$<0.001), and BCS ($P$<0.005; Figure 5.1), but not WH ($P$>0.159) or ADG ($P$>0.780; Supplementary Articles). Ewes in the control group had the lowest CG (92.4 cm), with highest CG of 97.6 cm observed in control group wethers. Mean BL of control group ewes (64.5 cm) was lower than all the other lambs, regardless of treatment or sex, which did not differ. Similarly, control group ewes had lower BCS (3.1) compared to all other treatment groups, except wethers on 10% *Spirulina* levels (3.3). Control group wethers had the greatest BWT (43.4 kg) and paradoxically control group ewes had the least BWT (37.9 kg). Between 10% and 20% *Spirulina* level groups there were no significant differences in BWT. However, ewes given 20% *Spirulina* levels had lower mean BWT than ewes receiving 10% (40.4 kg and 42.1 kg respectively).

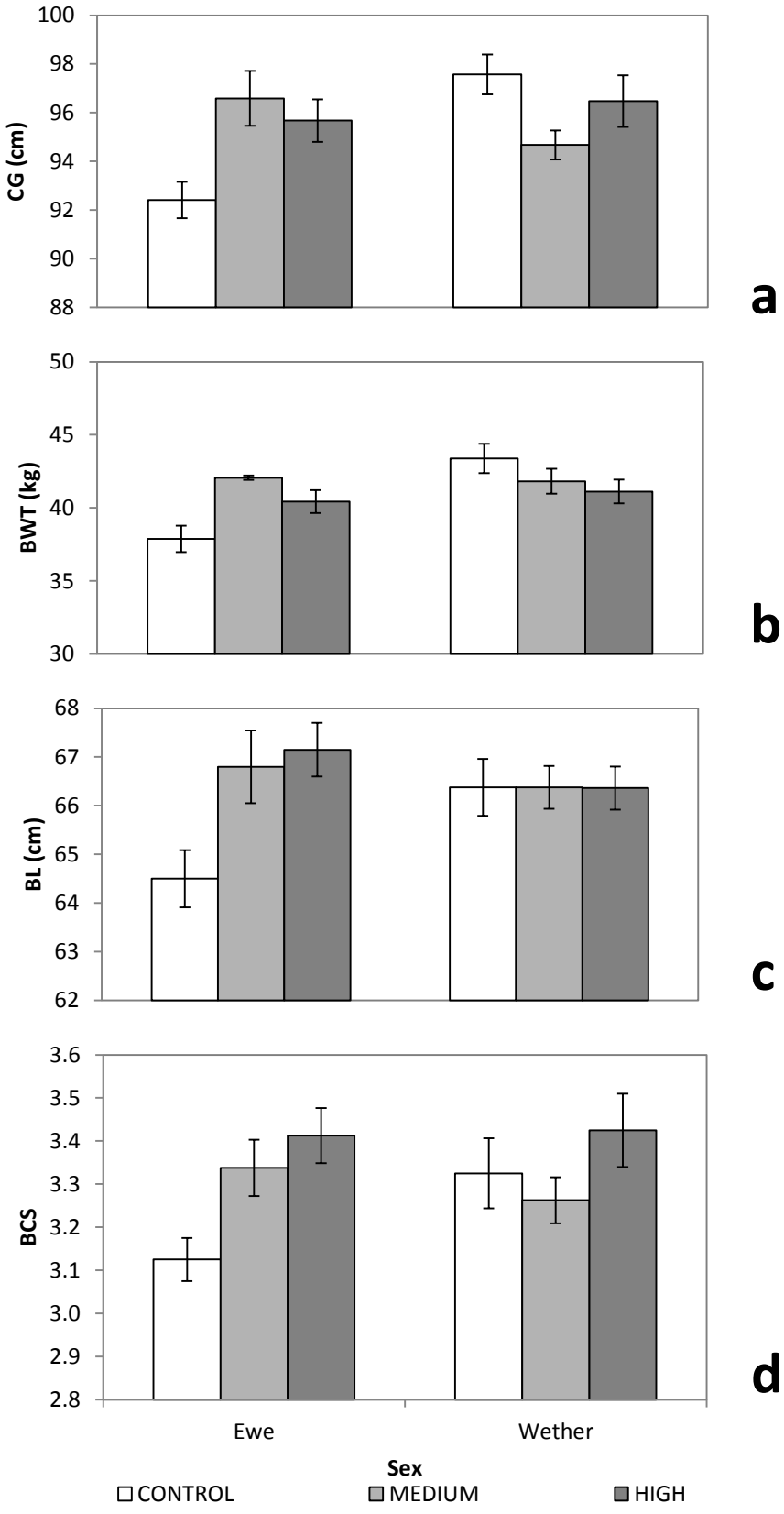


**Figure 5.6** *Spirulina* supplementation level and sex interactions on: a) chest girth (CG; *P*<0.001); b) liveweight (BWT; *P*<0.001); c) body length (BL; *P*<0.001); and d) body condition score (BCS; *P*<0.005).

*Phenotypic relationships between growth and body conformation traits*

Positive and significant correlations between CG, WH, BL, BCS and BWT were observed (*P*<0.001; Table 5.4). The strongest correlation of 0.83 was between BL and BWT. Only ADG had negligible correlations with all the other body conformation traits except BWT (*P*>0.05).

**Table 5.10** Liveweight, body conformation and average daily gains under typical plane of nutrition Pearson's correlation coefficients [1, 2]

| | WH (cm) | BL (cm) | BCS (1-5) | BWT (kg) | ADG (kg/day) |
|---|---|---|---|---|---|
| CG (cm) | 0.68*** | 0.59*** | 0.60*** | 0.59*** | -0.02$^{NS}$ |
| WH | | 0.64*** | 0.55*** | 0.63*** | 0.13$^{NS}$ |
| BL | | | 0.55*** | 0.83*** | 0.06$^{NS}$ |
| BCS | | | | 0.67*** | 0.01$^{NS}$ |
| BWT | | | | | 0.15* |

[1] Level of significant: $^{NS}$ no significance (*P*>0.05), * significant (*P*<0.05), *** very highly significant (*P*<0.001)
[2] Chest girth (CG), withers height (WH), body length (BL), body condition score (BCS), body weight (BWT), and average daily weight gain (ADG)

**Discussion**

The results in this study demonstrated that through *Spirulina* supplementation, liveweight and body conformation measurements can be better improved and managed under a typical pasture-based system. Moreover, *Spirulina* supplementation level interacted significantly with lamb sire breed and sex. This permits targeted management practises to be refined to suit different operational systems. To optimize growth and liveweight, lambs must have access to high quality feeds, particularly protein-rich feed supplements (Karlsson and, Martinsson, 2011; Liu *et al.*, 2003; Mitchell, 2007). *Spirulina*'s 60-70% protein content (Belay et al., 1993; Doreau et al., 2010; Mata et al., 2010) suggests that its increased use as a supplement in ruminants is expected to result in proportional improvements in lamb BWT, body conformations and ADG due to its association with increased rumen microbial crude protein production (Panjaitan et al., 2010; Quigley and, Poppi, 2009).

Our results demonstrate that the increase in liveweight and body condition score was only observed when the level of *Spirulina* supplementation was 10%. This is consistent with recently conducted trials with *Spirulina* (Bezerra et al., 2010) and other protein-rich supplement sources such as canola and lupins (Malau-Aduli et al., 2009a; Malau-Aduli et al., 2009b).

A possible explanation for this observation is that excessively high dietary protein intake is known to suppress optimal sheep growth. This effect stems from the negative correlation between protein accretion and fat deposition rates, the later being exacerbated by high feed protein levels (Mitchell, 2007). Excess protein gets deaminated and lost in the urine or gets broken down in the liver and could lead to conditions of fatty liver and ketosis. *Spirulina* contains many essential fatty acids including γ-linolenic acid (Bezerra et al., 2010) that get deposited subcutaneously (underneath the skin) as triacylglycerols in the adipose tissue, thus explaining the observed proportional increase of BCS with increased *Spirulina* supplementation levels. However, the insignificant influence of *Spirulina* level on ADG, CG and WH in the current study suggests that the physiological mechanisms involved are not fully understood at the moment. Thus, clarification of nutrient partitioning into different tissues including muscle, adipose and wool would allow greater insight into the underlying mechanisms in this species since lamb productivity is a function of genetic and environmental interactions (Black, 1983; Oddy and, Sainz, 2002). Thus, prime lamb productivity depends on not only environmental factors such as feed ration quality and quantity, but also on genetic factors in terms of lamb sire breed and sex (Malau-Aduli et al., 2009e; Sobrinho *et al.*, 2003).

The observed discrepancy in CG, BL and BCS between ewe and wether lambs in the current study seems to be due to variation in mature sizes and/or involvement of the endocrine system (Lewis et al., 2006). It has been demonstrated recently that the high oestrogen level of ewe which is associated with the closure of bone growth plate, also causes the rapid stoppage of growth of female lambs than wethers (Sowande and, Sobola, 2008; Warner et al., 2007). Based on these reports, it is evident that that nutrient demands, absorption and partitioning in ewes is different

from those of wethers and results in the utilization of *Spirulina* for increasing liveweight and body conformation. Previous studies demonstrated that genetic variation between lambs influences nutrient partitioning and absorption despite the feeding of the same or identical rations (Cake et al., 2007; Hegarty *et al.*, 2006c; Lewis et al., 2006; Oddy and, Sainz, 2002; Wynn and, Thwaites, 1981).

In the current study, liveweight and body conformation responses to *Spirulina* supplementation in Merino-and Black Suffolk-sired lambs were in accordance with our experimental hypothesis, although *Spirulina* supplementation did not elicit the expected response in Dorset- and White Suffolk-sired lambs as was hypothesised. This discrepancy might be due to variation in genetic predisposition for muscle growth as opposed to body fat deposition (Allingham et al., 2006). Also, feed use efficiency is enhanced by sire genetics as previously demonstrated (Mitchell, 2007; Ponnampalam et al., 2007). For instance, Lewis (2006) found that lambs whose parents were selected for high growth estimated breeding values grew larger than lambs whose lineage was selected for low growth, despite identical basal nutritional levels. Furthermore, the positive response of Merino-sired lambs to *Spirulina* supplementation levels is particularly worth mentioning as they were the only purebred and wool specialist group investigated in current study. Therefore, we can infer from our result that *Spirulina*'s growth promoting qualities in non-meat type sheep breeds and in combination with heterosis due to crossbreeding (Leymaster, 2001; Petrovic et al., 2011) does not impact the nutrient use efficiency or partitioning in the first crosses.

The result of current study confirmed the widely accepted interaction between sire breed and sex on liveweight and body conformation measurements, the basis of which has been extensively studied in the last decade (Afolayan et al., 2006; Cam et al., 2010; Fogarty et al., 2005a; Fogarty et al., 2005b; Hopkins et al., 2007b; Ponnampalam et al., 2007). Thus, a demonstration of the strong positive correlations between body measurements and liveweight in our present study reaffirms current consensus (Abbasi and, Ghafouri-Kesbi, 2011; López-Carlos et al., 2010; Otoikhian *et al.*, 2008; Sowande and, Sobola, 2008). Based on previous reports and our current

findings, it is therefore imperative to include body conformation measurements in selective breeding programs aimed towards increasing lamb liveweight (Abbasi and, Ghafouri-Kesbi, 2011; Cornelius and, Kaneko, 1963). Additionally, body conformation measurements would allow selection to prioritize growth of particular anatomical areas in lamb which would certainly improve the lamb productivity.

**Conclusion**

We have demonstrated herein, that both purebred Merino and Black Suffolk-sired crossbred lambs achieved higher liveweights and body conformation measurements with *Spirulina* supplementation than control lambs. Furthermore, 10% *Spirulina* supplementation levels resulted in increased liveweights over and above that observed in the control and HIGH *Spirulina* treatment groups. The linear increase in liveweight and body conformation measurements response to *Spirulina* supplementation observed in ewes was not reflected in wethers. These outcomes strongly recommend the establishment of *Spirulina* usage as an alternative sheep supplementary feed source for growth improvement as a strategic pathway for the early attainment of market weights in prime lambs.

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Chapter 6

# Effects of *Spirulina* supplementation on haematological metabolites in crossbred and purebred Australian Merino lambs

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**ABSTRACT**

This study investigated the effect of supplementing purebred and crossbred Merino lambs with *Arthrospira platensis* (*Spirulina*) on plasma metabolite concentrations under pasture-based management system and evaluated the influence of lamb sire breed and sex. The hypothesis tested was that *Spirulina supplementation will not be detrimental to the health and productivity of crossbred and purebred Merino lambs as indicated by haematological metabolite and electrolyte profiles, but significant interactions between level of supplementation, sire breed and sex will be the key drivers of variation*. A complete randomized block experimental design balanced by 4 sire breeds (Merino, White Suffolk, Dorset and Black Suffolk), 3 *Spirulina* supplementation levels (0, 100 and 200ml representing the CONTROL, MEDIUM and HIGH, respectively) and 2 sexes (ewe and wether lambs) was utilised. All lambs had *ad libitum* access to the basal diet of ryegrass pastures and barley grain. The *Spirulina* powder was dissolved in water and the lambs orally drenched daily for nine weeks. Lambs in the MEDIUM and HIGH *Spirulina* treatment groups were individually drenched daily with *Spirulina* solution prior to being released with the CONTROL group of lambs into paddocks for grazing over a 6-week period following a 3-week adjustment phase. At the completion of the feeding trial, blood samples were taken, serum separated from the plasma by centrifugation and analysed for the following

haematological metabolite and electrolyte concentrations: Creatine kinase, Aspartate aminotransferase (AST), glutamate dehydrogenase, gamma-glutamyl transferase (GGT), total bilirubin, creatinine, urea, protein, albumin, globulin, albumin to globulin ratio (A/G ratio), beta-hydroxybutyrate, glucose, calcium, magnesium, phosphate, sodium, potassium, sodium to potassium ratio, chloride, non-esterified fatty acids and serum cortisol. Data were analysed using SAS with *Spirulina* supplementation level, sire breed, sex and their second-order interactions fitted as fixed effects and metabolite concentrations as dependent variables. GGT concentrations decreased (from 79.40 to 69.25 UI) and glucose increased (from 3.81 to 4.19 mmol/L) as the level of *Spirulina* supplementation increased from 0ml in the CONTROL to 200ml in the HIGH treatment groups ($P<0.05$). Lambs supplemented at MEDIUM *Spirulina* levels had the highest creatinine concentrations (61.75µmol/L). Sex and *Spirulina* supplementation level interactions significantly affected glucose, AST and magnesium concentrations ($P<0.05$), while sire breed and *Spirulina* supplementation level interactions influenced A/G ratio, creatinine and GGT concentrations. These findings conclusively demonstrate that *Spirulina* supplementation does not negatively impact lamb health and productivity, hence the acceptance of the tested hypothesis.

(**Keywords**: *Arthrospira platensis, protein-rich supplement, plasma metabolites, electrolytes*)

**Introduction**

*Spirulina* (*Arthrospira platensis*) is a filamentous cyanobacterium which has been the recent subject of several feeding trials with agriculturally significant animal species (Holman and, Malau-Aduli, 2012b). However, to the best of our knowledge, published information regarding the metabolite response of crossbred and Merino purebred lambs to *Spirulina* supplementation remains relatively scarce.

In Australia, crossbred Merino lambs are generally a product of dual-purpose sheep production systems. These systems typically mate meat-type rams with a core

Merino flock to introduce both desirable meat and wool traits into the subsequent progeny (Fogarty et al., 2006). Resultant lambs are routinely supplemented with protein-rich feed types to optimise growth and productivity (Kopke et al., 2008). Consequently, dual-purpose producers are best situated to exploit the current high lamb meat prices (ABARE., 2012) without abandoning their traditional wool interests. In dual-purpose systems, as in other sheep producing systems, lamb health is equally as important as productivity.

Knowledge of haematological metabolite concentrations is valuable in understanding individual lamb health and productivity status (Braun et al., 2010; Russell and, Roussel, 2007). Hence, quantifying key haematological metabolite concentrations has been applied to measure the response of lambs to alternative diets and feed supplements (Hatfield *et al.*, 1998b; Hegarty et al., 2006b). We hypothesised that health and productivity as indicative by haematological metabolites in the plasma of crossbred and purebred Merino lambs would be beneficially altered with *Spirulina* supplementation.

Our aim was to investigate the effects of *Spirulina* supplementation at differing levels to crossbred and purebred Merino lambs on haematological metabolites as indicators of health and productivity. We also evaluated the existing interactions of *Spirulina* supplementation levels with sire breed and sex.

**Materials and Methods**

This study was conducted at the University Farm, Cambridge TAS, and was approved by the Tasmanian Animal Ethics Committee in accordance with the 1993 Tasmania Animal Welfare Act and the 2004 Australian code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004).

*Animal management and experimental design*

Using a 1:100 mating ratio, approximately 1600 Merino ewes were mated with 16 terminal sire rams of different breeds – Black Suffolk, Dorset, Merino, and White Suffolk. All progeny were identified with National Livestock Identification ear tags before being weaned onto ryegrass pasture at 16 weeks of age. At 6 months old, 24 lambs were randomly allotted into a completely randomised block experimental design balanced by sire breed, *Spirulina* supplementation levels and sex, respectively.

The *Spirulina* was commercially purchased (TAAU, Darwin, AUS) as a powder (Table 6.1) which was then made into a water suspension using a *Spirulina* to water ratio of 1g:10mL. This was daily given to the lambs using a sheep drench to directly deliver each lamb's assigned *Spirulina* level of supplementation – CONTROL (0mL), MEDIUM (100mL), and HIGH (200mL). Supplementation continued over the 9-week feeding trial duration, consisting of a 3-week adjustment phase and 6-week experimental period (Trenkle, 1978). All experimental lambs were run together as a single mob with *ad libitum* access to drinking water and a basal diet of ryegrass pasture and barley grain.

**Table 6.11** Nutrient composition of *Spirulina* and basal diet of ryegrass pasture and barley [1]

| *Chemical composition* | **Feed components** | | | *Unit* |
|---|---|---|---|---|
| | *Spirulina* | Barley grain | Ryegrass pasture | |
| Moisture[2] | 4.0 | 6.8 | 55.3 | g/100g Fresh Wt |
| DM | 96.0 | 93.2 | 44.7 | g/100g Fresh Wt |
| NDF | 32.6 | 18.5 | 22.4 | g/100g DM |
| NDFn[3] | 30.3 | 17.2 | 20.8 | g/100g DM |
| ADF | 18.3 | 6.0 | 23.0 | g/100g DM |
| NFC[4] | 7.9 | 68.7 | 43.5 | g/100g DM |
| Ash | 9.5 | 3.2 | 11.9 | g/100g DM |
| EE | 5.9 | 2.0 | 3.0 | g/100g DM |
| CP | 62.2 | 8.9 | 20.8 | g/100g DM |
| ME[5] | 1707.5 | 1723.7 | 1701.1 | kJ/100g DM |

[1] Dry matter (DM), neutral detergent fibre (NDF), nitrogen free NDF (NDFn), non fibrous carbohydrate (NFC), acid detergent fibre (ADF), ether extract (EE), indigestible organic matter (IOM), and metabolisable energy (ME)
[2] Moisture = 100 – DM
[3] NDFn = NDF x 0.93 (Undersander and, Moore, 2002)
[4] NFC = 100 – (NDFn + CP + EE + Ash) (Undersander and, Moore, 2002)
[5] ME = 4194 – (9.2 x Ash) + (1.9 x CP) + (3.9 x EE) – (3.5 x NDF) (Noblet and, Perez, 1993)

*Blood sampling and analysis*

Blood samples were taken using jugular venipuncture (Long *et al.*, 1965b) at the completion of the feeding trial. These were stored in BA Vacutainer® tubes without anticoagulant (Becton, Dickson and Company, Belliver Industrial Estate, Plymouth, UK), before being immediately chilled before being centrifuged at 3000 rpm for 20 minutes at 4°C (Ponnampalam *et al.*, 2005). Sub-samples of plasma and serum were taken and stored at -20°C until analysis (Russell and, Roussel, 2007).

All samples were commercially analysed for haematological metabolite concentrations at the Animal Health Laboratory of the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE, Launceston, AUS) using kits (Thermo Scientific) for all metabolites excluding GLDH which was supplied by Randox). Specifically, creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), total bilirubin, creatinine, urea, protein, albumin, globulin, albumin to globulin ratio (A/G ratio), beta-hydroxybutyrate (BHB), glucose, calcium, magnesium, phosphate, sodium, potassium, sodium to potassium ratio (Na/K ratio), and chloride concentrations were analysed on a Konelab 20XTi Clinical Chemistry Analyser (Thermo Scientific).

Non-esterified fatty acids (NEFA) were measured enzymatically by Regional Laboratory Services (Benalla, Victoria, AUS) in a sample blanked endpoint reaction (Randox Laboratories, Crumlin, UK, product #FA 115) via Acyl CoA Synthetase/ Acyl CoA Oxidase/ Peroxidase as per Matsubara et al. (1983). Serum cortisol was commercially tested by Gribbles Pathology (Clayton, Victoria, AUS) using an Immulite 2000 Systems analyser (Siemens; GERMANY) and a solid-phase, competitive chemiluminescent enzyme immunoassay.

*Statistical analysis*

All data were analysed using 'Statistical Analysis System' software (SAS Institute., 2009). Summary statistics were initially computed and the unadjusted means,

standard deviations, minimum and maximum values scrutinised for outliers and data entry errors. Data were then further analysed using Factorial ANOVA (PROC GLM) procedures with *Spirulina* supplementation level, sire breed, sex and their second-order interactions fitted as fixed effects and the following haematological metabolites as dependent variables: CK, AST, GLDH, GGT, bilirubin, creatinine, urea, protein, albumin, globulin, A/G ratio, BHB, glucose, calcium, magnesium, phosphate, sodium, potassium, Na/K ratio, chloride, NEFA, serum cortisol. Both Duncan's multiple range and Bonferroni pairwise comparison tests were used for mean separation using a $P<0.05$ level of significance.

**Results**

Chemical compositions of the *Spirulina* supplement and the basal diet of ryegrass pasture and barley grain are shown in Table 1, wherein *Spirulina* is protein-rich (61.0 g/100g DM) accounting for twice as much protein in the combined basal diet. In terms of metabolisable energy, *Spirulina* and the basal diets were iso-energetic ranging from 1701.1-1723.7 kJ/100g DM. Dry matter and crude fibre contents were sufficient to meet lamb gut-fill requirements.

*Effect of Spirulina supplementation level on haematological metabolites*

GGT concentrations were highest in unsupplemented (CONTROL) lambs compared to their supplemented counterparts in the MEDIUM and HIGH treatment groups (Table 6.2). Lambs supplemented at the MEDIUM *Spirulina* level had the highest creatinine concentrations averaging 61.75 ± 1.72 µmol/L. Glucose concentrations were highest in lambs supplemented at the HIGH *Spirulina* level (Table 6.2).

**Table 6.12** *Spirulina* supplementation level independent affect on haematological metabolite least square means and standard error (LSM ± SE) [1, 2]

| | *Spirulina* supplementation level | | | | |
|---|---|---|---|---|---|
| | **Control** | **Low** | **High** | *Normal range* | Units |
| CK | 297.44 ± 31.54 | 249.00 ± 15.08 | 310.88 ± 22.43 | 130 – 350 | UI |
| AST | 117.94 ± 5.26 | 130.06 ± 9.28 | 129.40 ± 10.91 | 0 – 220 | UI |
| GLDH | 15.56 ± 3.50 | 25.81 ± 7.48 | 23.27 ± 5.91 | 0 – 41 | UI |
| GGT | 79.40 ± 2.43$^{A}$ | 70.81 ± 2.98$^{B}$ | 69.25 ± 3.04$^{B}$ | 31 – 72 | UI |
| Total Bilirubin | 3.11 ± 0.20 | 3.28 ± 0.18 | 3.06 ± 0.14 | 0 – 13 | µmol/L |
| BHB | 0.37 ± 0.02 | 0.43 ± 0.02 | 0.43 ± 0.03 | 0.0 – 0.8 | mmol/L |
| Creatinine | 57.19 ± 1.61$^{B}$ | 61.75 ± 1.72$^{A}$ | 58.81 ± 1.43$^{B}$ | 69 – 168 | µmol/L |
| Urea | 7.72 ± 0.31 | 7.99 ± 0.40 | 7.79 ± 0.32 | 2.8 – 7.2 | mmol/L |
| Protein | 64.46 ± 1.16 | 66.08 ± 1.24 | 64.12 ± 0.85 | 60 – 82 | g/L |
| Albumin | 35.53 ± 0.57 | 36.30 ± 0.53 | 35.73 ± 0.50 | 24 – 30 | g/L |
| Globulin | 27.50 ± 1.38 | 29.75 ± 1.05 | 29.00 ± 1.21 | 35 – 57 | g/L |
| A/G Ratio | 1.25 ± 0.05 | 1.24 ± 0.04 | 1.28 ± 0.05 | 0.6 – 1.3 | . |
| Glucose | 3.81 ± 0.09$^{B}$ | 4.04 ± 0.12$^{AB}$ | 4.19 ± 0.16$^{A}$ | 2.77 – 4.44 | mmol/L |
| NEFA | 0.82 ± 0.07 | 0.76 ± 0.09 | 0.82 ± 0.06 | 0.20 – 0.80 | mmol/L |
| Cortisol | 62.88 ± 12.36 | 55.56 ± 9.25 | 52.25 ± 8.40 | 50.5 – 70.5 | nmol/L |
| | | | | | |
| Calcium | 2.51 ± 0.03 | 2.52 ± 0.03 | 2.54 ± 0.04 | 2.4 – 3.2 | mmol/L |
| Magnesium | 0.95 ± 0.02 | 0.94 ± 0.02 | 0.91 ± 0.01 | 0.82 – 1.23 | mmol/L |
| Phosphate | 2.02 ± 0.07 | 1.93 ± 0.06 | 1.99 ± 0.07 | 1.61 – 2.35 | mmol/L |
| Sodium | 142.06 ± 0.32 | 142.44 ± 0.32 | 142.19 ± 0.21 | 139 – 152 | mmol/L |
| Potassium | 4.80 ± 0.09 | 4.72 ± 0.10 | 4.63 ± 0.05 | 3.9 – 5.4 | mmol/L |
| Na/K Ratio | 29.81 ± 0.54 | 30.50 ± 0.63 | 30.88 ± 0.40 | . | . |
| Chloride | 106.06 ± 0.36 | 105.81 ± 0.55 | 106.25 ± 0.54 | 95 – 103 | mmol/L |

[1] Row means bearing different superscripts significantly differ ($P$<0.05)
[2] Creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), beta-hydroxybutyrate (BHB), albumin/globulin ratio (A/G Ratio), non esterfied fatty acids (NEFA)

*Comparison between haematological metabolite concentration and normal range*

Urea, albumin and chloride concentrations exceeded their normal ranges in both supplemented and unsupplemented lambs. In contrast, creatinine and globulin concentrations were below their normal ranges in all supplemented and CONTROL lambs (Table 6.2).

*Interaction effects between Spirulina supplementation level and sire breed on haematological metabolites*

Creatinine concentrations were highest in Dorset-sired lambs supplemented at MEDIUM *Spirulina* level. Merino-sired lambs supplemented at the MEDIUM *Spirulina* level had lowest creatinine concentrations (Figure 6.1a). A/G ratios were higher in Black Suffolk- and Dorset-sired lambs supplemented at the HIGH *Spirulina* level compared to their counterparts supplemented at the MEDIUM level. Purebred Merino lambs supplemented at the MEDIUM *Spirulina* level had the highest A/G ratios (Figure 6.1b). GGT concentrations were highest in unsupplemented (CONTROL) purebred Merino lambs. In White Suffolk-sired lambs, GGT concentrations progressively decreased with increase in *Spirulina* supplementation levels (Figure 6.1c; Supplementary Articles).

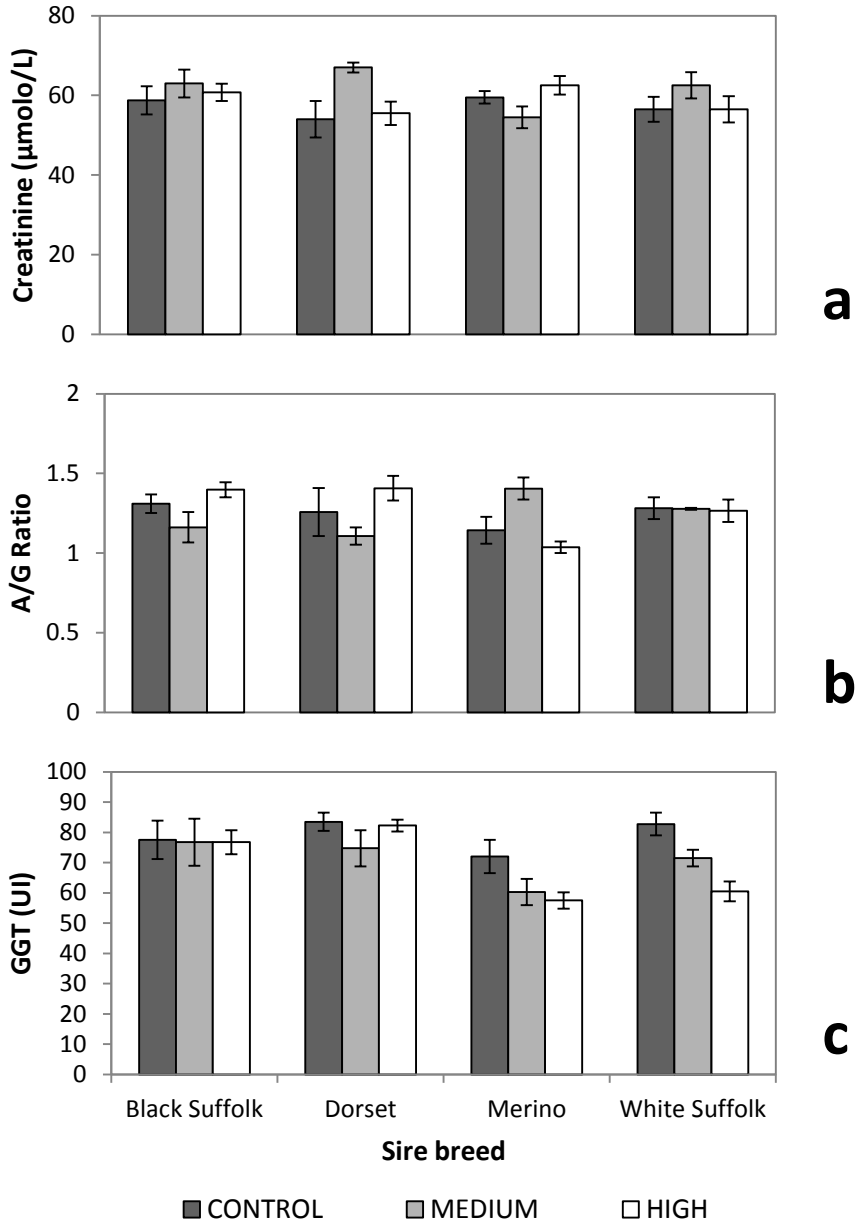


**Figure 6.7** *Spirulina* supplementation level and sire breed interactions on: a) creatinine ($P$<0.001); b) albumin/globulin ratio (A/G Ratio; $P$<0.007); and c) gamma-glutamyl transferase (GGT; $P$<0.010).

*Spirulina supplementation level and sex interactions effect on haematological metabolites*

Glucose and AST concentrations were lowest in CONTROL ewe lambs (Figure 6.2a). Wether AST concentrations were lowest with HIGH *Spirulina* supplementation levels (Figure 6.2b). Ewe lambs supplemented at MEDIUM *Spirulina* levels and wether lambs in the HIGH supplementation treatment group had the highest Mg concentrations (Figure 6.2c; Supplementary Articles).

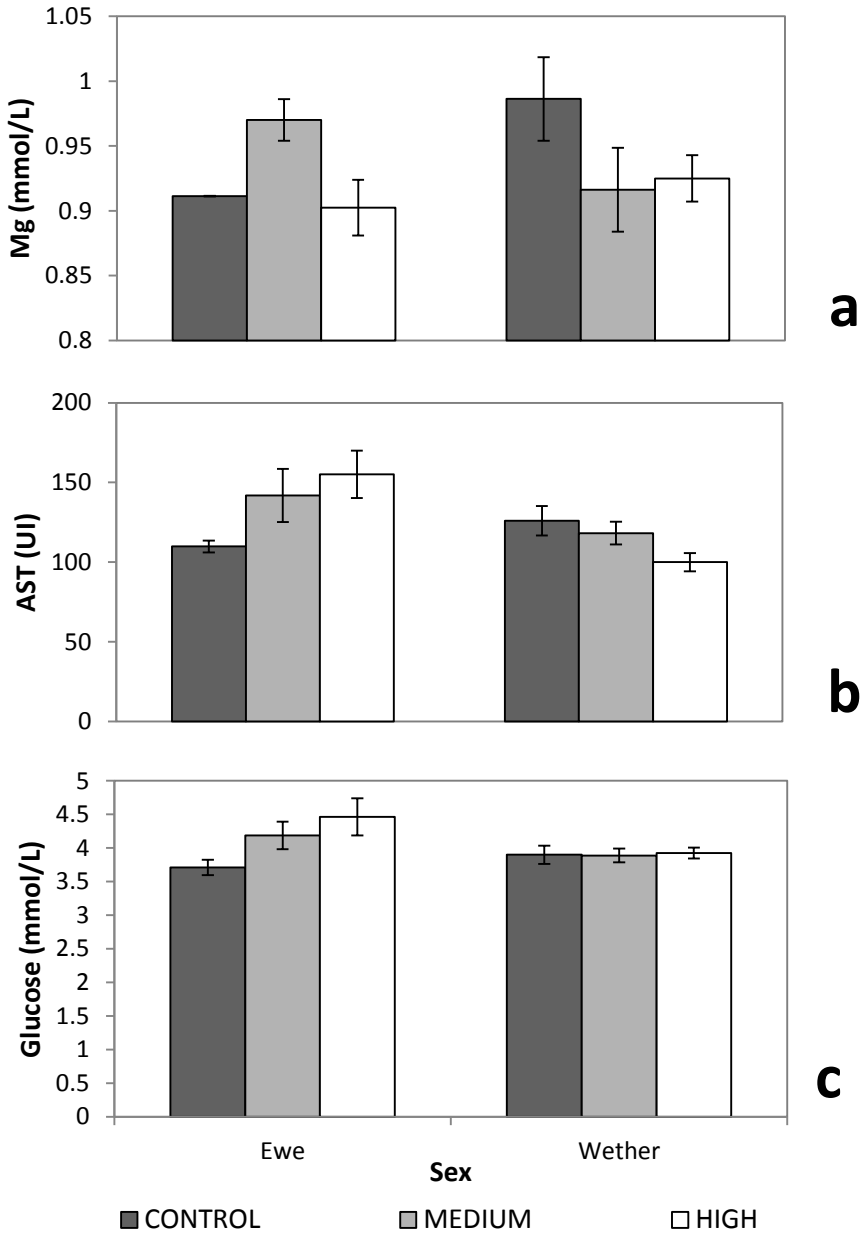


**Figure 6.8** *Spirulina* supplementation level and sex interactions on: a) magnesium (*P*<0.050); b) aspartate aminotransferase (AST; *P*<0.012); and c) glucose (*P*<0.020).

*Sire breed and sex interactions effect on haematological metabolites*

Black Suffolk-sired lambs had the lowest creatinine and urea concentrations while Merino-sired lambs had the lowest albumin concentrations. Dorset-sired lambs had higher glucose concentrations than White Suffolk-sired lambs. Chloride concentrations were higher in Dorset- and White Suffolk-sired lambs than Merino (Table 6.3). GGT and glucose concentrations were higher in ewes than wethers, but Creatinine and protein concentrations were highest in wethers (Table 6.3). Dorset-sired ewes had higher glucose concentrations than their wether counterparts (Figure 6.3a). In Black Suffolk-sired lambs, ewes had higher GGT concentration than wethers (Figure 6.3b; Supplementary Articles).

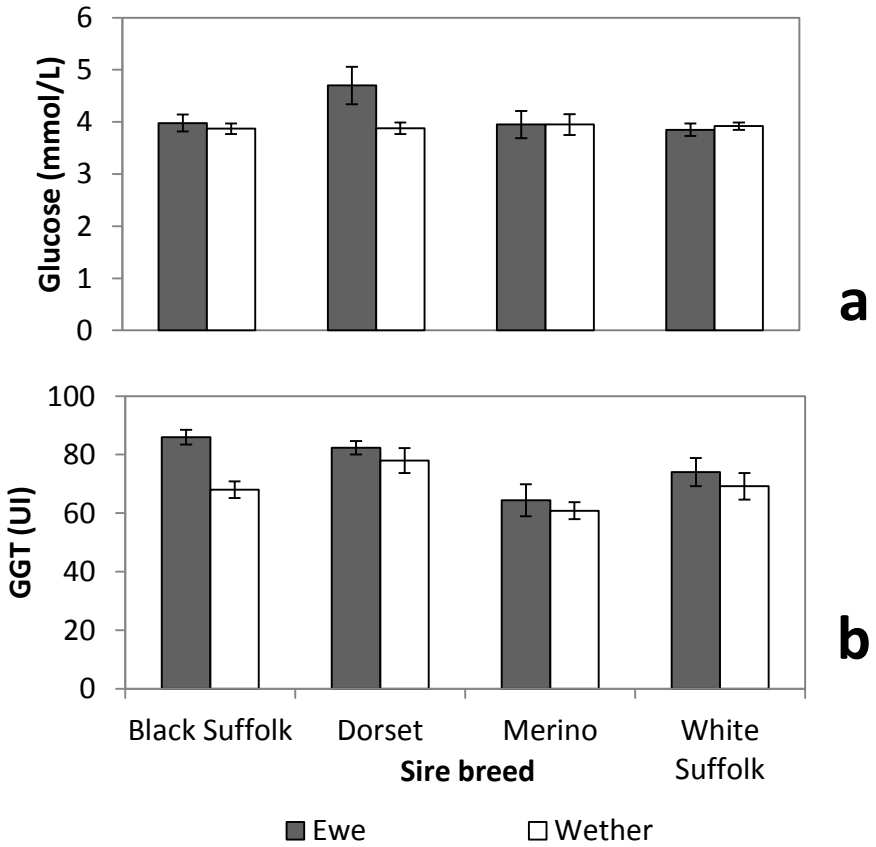


**Figure 6.9** Sire breed and sex interactions on: a) glucose (P<0.016); and b) gamma-glutamyl transferase (GGT; P<0.027).

**Table 6.13** Sire breed and sex independent effects on haematological metabolites least square means and standard error (LSM ± SE) [1, 2]

| | Sire breed | | | | Sex | | | |
|---|---|---|---|---|---|---|---|---|
| | **Black Suffolk** | **Dorset** | **Merino** | **White Suffolk** | **Ewe** | **Wether** | *Normal range* | *Units* |
| CK | 275.75 ± 30.61 | 332.00 ± 37.89 | 248.75 ± 138.75 | 294.33 ± 25.35 | 283.78 ± 23.57 | 289.67 ± 16.89 | 130 – 350 | UI |
| AST | 119.00 ± 10.35 | 125.50 ± 9.89 | 138.75 ± 10.21 | 119.08 ± 9.65 | 135.63 ± 8.22 | 115.39 ± 8.22 | 0 – 220 | UI |
| GLDH | 27.64 ± 8.72 | 19.42 ± 6.20 | 17.83 ± 4.17 | 21.67 ± 7.91 | 23.67 ± 5.52 | 19.26 ± 3.87 | 0 – 41 | UI |
| GGT | 77.00 ± 3.26 | 80.17 ± 2.40 | 62.45 ± 2.84 | 71.58 ± 3.23 | $77.22 \pm 2.50^{A}$ | $69.00 \pm 2.15^{B}$ | 31 – 72 | UI |
| Total Bilirubin | 2.98 ± 0.15 | 3.59 ± 0.27 | 3.11 ± 0.17 | 2.92 ± 0.15 | 3.13 ± 0.16 | 3.17 ± 0.12 | 0 – 13 | µmol/L |
| BHB | 0.44 ± 0.03 | 0.37 ± 0.03 | 0.42 ± 0.03 | 0.40 ± 0.03 | 0.43 ± 0.02 | 0.39 ± 0.02 | 0.0 – 0.8 | mmol/L |
| Creatinine | 60.83 ± 1.72 | 58.83 ± 2.43 | 58.83 ± 1.54 | 58.50 ± 1.89 | $57.08 \pm 1.43^{B}$ | $61.42 \pm 1.08^{A}$ | 69 – 168 | µmol/L |
| Urea | $7.19 \pm 0.42^{B}$ | $7.50 \pm 0.25^{AB}$ | $8.18 \pm 0.49^{AB}$ | $8.47 \pm 0.28^{A}$ | 7.63 ± 0.27 | 8.04 ± 0.29 | 2.8 – 7.2 | mmol/L |
| Protein | 64.73 ± 1.29 | 66.05 ± 1.29 | 62.88 ± 1.02 | 65.89 ± 1.38 | $63.32 \pm 0.79^{B}$ | $66.45 \pm 0.90^{A}$ | 60 – 82 | g/L |
| Albumin | $36.23 \pm 0.38^{A}$ | $36.385 \pm 0.50^{A}$ | $33.96 \pm 0.62^{B}$ | $36.84 \pm 0.61^{A}$ | 35.44 ± 0.47 | 36.26 ± 0.36 | 24 – 30 | g/L |
| Globulin | 27.50 ± 0.92 | 29.50 ± 2.00 | 28.67 ± 1.38 | 29.33 ± 1.33 | 28.47 ± 0.95 | 29.08 ± 1.06 | 35 – 57 | g/L |
| A/G Ratio | 1.29 ± 0.05 | 1.26 ± 0.07 | 1.20 ± 0.06 | 1.28 ± 0.03 | 1.29 ± 0.04 | 1.22 ± 0.03 | 0.6 – 1.3 | . |
| Glucose | $3.93 \pm 0.09^{AB}$ | $4.29 \pm 0.22^{A}$ | $3.95 \pm 0.16^{AB}$ | $3.88 \pm 0.06^{B}$ | $4.12 \pm 0.13^{A}$ | $3.90 \pm 0.06^{B}$ | 2.77 – 4.44 | mmol/L |
| NEFA | 0.90 ± 0.09 | 0.85 ± 0.08 | 0.77 ± 0.09 | 0.68 ± 0.07 | 0.83 ± 0.06 | 0.77 ± 0.06 | 0.20 – 0.80 | mmol/L |
| Cortisol | 76.08 ± 14.47 | 61.83 ± 14.11 | 49.58 ± 6.95 | 40.08 ± 6.78 | 54.38 ± 7.83 | 59.42 ± 8.61 | 50.5 – 70.5 | nmol/L |
| | | | | | | | | |
| Calcium | 2.50 ± 0.04 | 2.53 ± 0.04 | 2.55 ± 0.02 | 2.50 ± 0.04 | 2.50 ± 0.02 | 2.54 ± 0.03 | 2.4 – 3.2 | mmol/L |
| Magnesium | 0.94 ± 0.02 | 0.96 ± 0.02 | 0.92 ± 0.02 | 0.93 ± 0.02 | 0.93 ± 0.01 | 0.94 ± 0.02 | 0.82 – 1.23 | mmol/L |
| Phosphate | 1.95 ± 0.08 | 2.03 ± 0.08 | 1.81 ± 0.06 | 2.13 ± 0.04 | 1.95 ± 0.05 | 2.01 ± 0.06 | 1.61 – 2.35 | mmol/L |
| Sodium | 142.42 ± 0.31 | 142.08 ± 0.31 | 142.08 ± 0.31 | 142.33 ± 0.40 | 142.50 ± 0.24 | 141.96 ± 0.21 | 139 – 152 | mmol/L |
| Potassium | 4.68 ± 0.11 | 4.74 ± 0.08 | 4.61 ± 0.08 | 4.83 ± 0.12 | 4.68 ± 0.08 | 4.75 ± 0.06 | 3.9 – 5.4 | mmol/L |
| Na/K Ratio | 30.67 ± 0.66 | 30.08 ± 0.53 | 31.17 ± 0.56 | 29.67 ± 0.56 | 30.67 ± 0.50 | 30.13 ± 0.35 | . | . |
| Chloride | $105.92 \pm 0.51^{AB}$ | $106.92 \pm 0.71^{A}$ | $104.75 \pm 0.25^{B}$ | $106.58 \pm 0.50^{A}$ | 106.21 ± 0.40 | 105.88 ± 0.39 | 95 – 103 | mmol/L |

[1] Row means within independent effects bearing different superscripts significantly differ ($P<0.05$)

[2] Creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), beta-hydroxybutyrate (BHB), albumin/globulin ratio (A/G Ratio), non esterfied fatty acids (NEFA)

**Discussion**

Although significant differences attributable to level of *Spirulina* supplementation were only detected in GGT, creatinine and glucose profiles, there are vital animal welfare and well-being implications for the non-significant differences in cortisol, protein, albumin, globulin, urea and NEFA concentrations. It has been demonstrated that cortisol is an indicator of stress levels (Bradshaw et al., 1996). The fact that lambs supplemented with *Spirulina* did not have elevated cortisol levels compared to the control lambs implies that oral drenching with *Spirulina* supplement did not trigger discomfort to the lambs nor did it compromise their welfare. Globulin, albumin, urea are all directly related to protein metabolism and their concentrations in both supplemented and CONTROL animals fell within the normal range (Table 6.2). Normality was also evident in the electrolyte concentrations of Ca, P, Mg, Na and K indicating that mineral metabolism was not negatively impacted by *Spirulina* supplementation.

However, level of *Spirulina* supplementation had direct impact on GGT, creatinine and glucose concentrations (Table 6.2) and interacted significantly with sire breed and sex to influence metabolites and electrolytes in lambs as depicted in Figures 6.1-6.3. These are discussed in some more detail below:

*Spirulina supplementation decreased gamma-glutamyl transferase concentrations*

GGT plays a critical role in cellular detoxification and is found in the kidneys, pancreas, intestine and liver (Center, 2007). The liver is the main source of GGT (Russell and, Roussel, 2007). Hence, haematological GGT is a useful biomarker of optimal liver function (Byrne *et al.*, 2012). In sheep, haematological GGT concentrations will elevate from normal ranges during periods of hepatic tissue damage, injury and disease (Braun et al., 2010). Our results suggest that *Spirulina* supplementation amended elevated GGT concentrations towards normality. Hence, *Spirulina* supplementation can be associated with improved lamb liver health. Other

researchers have reaffirmed this association by linking *Spirulina* consumption with improved liver health (Belay et al., 1993; Colla *et al.*, 2008; Ismail *et al.*, 2009).

*Spirulina supplementation effects creatinine and glucose concentrations*

Assessment of haematological creatinine allows growth, muscularity and total body protein mass to be objectively determined (Hegarty et al., 2006b). This stems from the positive linear relationship between creatinine concentrations and muscle tissue mass. Therefore, lambs with high haematological creatinine concentrations have greater muscularity than others with lower creatinine concentrations. Holman et al. (2012) found low *Spirulina* supplementation levels resulted in higher lamb liveweights than in both control and high *Spirulina* supplementation level groups. This finding aligns with similar outcomes from supplementing sheep with soybean meal (Hatfield et al., 1998b), canola meal and cracked lupins (Kulpys et al., 2009; Malau-Aduli *et al.*, 2009d). This increase in liveweight, thus lamb muscularity, with low supplementation levels, is reflected in current study and associated with haematological creatinine concentration.

Ruminants do not absorb preformed dietary glucose in their guts (Russell and, Roussel, 2007). Instead, glucose requirements are met using gluconeogensis pathways sourcing carbon from complex carbohydrates (Cronje, 1990), lipids (Filipovic et al., 2011), and protein (Annison and, White, 1961). The observed change in haematological glucose with increased dietary protein, through *Spirulina* supplementation is confirmatory of this pathway. *Spirulina* supplementation would have also affected the dietary energy intake of lambs, which is directly related to haematological glucose concentrations (Kempton and, Leng, 1983). Trenkle (1970) found similar increases in haematological glucose concentrations of supplemented lambs on a basal diet of grains as opposed to *Spirulina*. However, glucose concentrations rapidly and frequently change dependent on individual lamb and other factors (Filipovic et al., 2011).

*Spirulina supplementation level and sex interactions effects on glucose, aspirate amino transferase and magnesium concentrations*

Haematological glucose concentrations have been shown to have a negative linear association with lamb liveweight (Kempton and, Leng, 1983) and wethers have been reported to have higher liveweights (Holman et al., 2012) and muscularity (Geesink and, Zerby, 2010) than ewes under identical diets. In this study, it can be suggested that dietary protein in these experimental lambs was more likely partitioned more towards total body protein mass stores rather than gluconeogenesis in wethers compared to ewes. Furthermore, as AST catalyses gluconeogenesis (Center, 2007; Vernon *et al.*, 1987), its response to *Spirulina* supplementation level and sex interactions is identical to haematological glucose concentration.

Magnesium concentrations have been shown to increase proportionally with growth (Long *et al.*, 1965a). Hence, this finding can be attributable to the liveweight responses to *Spirulina* supplementation level and sex interactions observed previously (Holman et al. (2012). Moreover, *Spirulina* has a high concentration of calcium (Holman and, Malau-Aduli, 2012b) and dietary calcium levels have been found to negate haematological magnesium concentrations (Ponnampalam et al., 2005). However, this relationship was only observed in wethers in this study.

*Spirulina supplementation level and sire breed interactions affect on gamma-glutamyl transferase and creatinine concentrations and albumin/globulin ratio*

It has been reported that haematological GGT concentrations naturally vary between lambs of differing sire breeds (Morris *et al.*, 2001; Phua *et al.*, 2009). This stems from different levels of disease resistance, liver functionality and response to dietary protein sources found between lambs of differing genotypes (Edrington *et al.*, 1994; Hatfield *et al.*, 1998a). This is confirmed in our current study given the genetically divergent sire breeds. Lamb sire breed has also been shown to affect lamb liveweight (Ponnampalam et al., 2007), and predisposition for muscle growth rather than fat deposition (Allingham et al., 2006). These can be attributed to influences on feed use

efficiency varying between sire breeds (Scales et al., 2000). This study found haematological creatinine concentrations reflecting these differences in liveweight and muscularity that we previously found between sire breeds supplemented with *Spirulina* (Holman et al., 2012).

Albumin and globulin are the main protein components of plasma which are typically monitored as A/G ratio (Russell and, Roussel, 2007). Haematological albumin concentrations are indicative of long term dietary protein intake (Sargison and, Scott, 2010). Consequently, lambs supplemented with HIGH *Spirulina* levels have greater long term dietary protein intakes which would influence albumin levels and, in term, alter the A/G ratios, as shown. Merino-sired lambs' divergence from other sire breed A/G ratio trends may have arisen from previously discussed differences in feed use efficiency.

*Sex and sire breed effects on haematological metabolites*

Wethers generally have greater liveweights and muscularity than ewes (Geesink and, Zerby, 2010; Lee *et al.*, 2001; Scales et al., 2000), hence the observed differences in haematological creatinine, protein and glucose concentrations between wethers and ewes in our current study. Sex interacts with sire breed to affect liveweight and muscularity mainly by influencing feed use efficiency (Hegarty et al., 2006b; Malau-Aduli et al., 2009a) and growth potential (Fogarty et al., 2005a; Sobrinho et al., 2003).

*Haematological metabolites outside normal ranges*

Creatinine concentration was outside the normal range and since it is associated with muscularity, our findings tend to suggest that this was expected as all experimental lambs were actively growing weaners whose liveweights and muscularity had not yet reached the levels expected in mature wethers and ewes.

Urea, the principal by-product of protein metabolism synthesised in the liver, is transported for excretion by the kidneys or for recycling in the gut (Russell and,

Roussel, 2007). Hence, haematological urea concentrations relate to dietary nitrogen supply (Huntington and, Archibeque, 2000) and dietary protein intake. Therefore, increased provision of protein through *Spirulina* supplementation would have induced the observed findings. However, haematological urea concentrations only represent short-term changes in dietary protein intake (Sargison and, Scott, 2010).

Haematological albumin concentrations are a function of dietary protein (Meijers et al., 2008). Furthermore, haematological globulin concentrations are typically calculated by subtracting albumin concentrations from total protein concentrations (Russell and, Roussel, 2007). This study found total protein concentrations to be within normal range, therefore observed globulin concentrations would have resulted from elevated albumin concentrations.

Haematological chloride concentrations are useful indicators of animal hydration (Russell and, Roussel, 2007). Also, these concentrations are only indicative of animal status at the time of sampling (Sargison and, Scott, 2010). Therefore, we can assume that the experimental lambs were probably slightly dehydrated during blood sampling.

**Conclusion**

Our findings demonstrate that *Spirulina* supplementation can lower haematological GGT concentrations by 10 UI on the average, compared to unsupplemented lambs, but this difference varies depending on interactions with lamb sire breed and sex. Creatinine levels were indicative of muscularity and lambs supplemented at low *Spirulina* levels had the highest muscularity. HIGH *Spirulina* supplementation levels resulted in the highest glucose concentrations indicative of available energy for driving protein metabolism and other metabolic pathways including gluconeogenesis. Furthermore, *Spirulina* supplementation, sex and sire breed interacted to affect glucose, AST, magnesium, A/G ratios, creatinine and GGT concentrations. These findings highlight the beneficial impact of *Spirulina* supplementation on lamb health and productivity, hence the acceptance of the tested hypothesis that *Spirulina*

supplementation of crossbred and purebred Merino lambs will not be detrimental to health and productivity as indicated by haematological metabolite and electrolyte profiles. The fact that significant interactions between level of supplementation, sire breed and sex were the key drivers of observed variation in metabolite profiles, gives dual-purpose sheep farmers the flexible options of choosing appropriate sire breeds to match the level of *Spirulina* supplementation for optimal lamb productivity and health.

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Chapter 7

# Modelling the feed intake of purebred and crossbred Merino lambs supplemented with *Spirulina*

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**ABSTRACT**

Several feed intake models that included residual feed intake (RFI), residual liveweight gain (RLG), standardised daily feed intake (SDFI), feed conversion ratio (FCR), metabolic feed conversion ratio ($FCR_{met}$), daily feed intake (DFI) and specific growth rate (SGR) were tested in purebred and crossbred Merino lambs. The aim was to evaluate the effects of *Spirulina* supplementation, sire breed, sex and their interactions on voluntary feed intake in Australian dual-purpose lambs fed a basal diet of lucerne hay and barley grains. Twenty four ewe and wether lambs belonging to 3 sire breeds (Dorset, Merino, White Suffolk), were randomly allocated to 4 Spirulina supplementation levels (CONTROL – 0ml, LOW – 50ml, MEDIUM – 100ml, HIGH – 200ml) in a nine-week feeding trial. Data were analysed in SAS using general linear model procedures, with sire breed, *Spirulina* supplementation level, sex and their second order interactions as fixed effects, sire as a random effect and feed intake as the dependent variable. Feed intake significantly differed between sire breeds with regard to DFI and SDFI models, but neither sex nor *Spirulina* supplementation level influenced feed intake in any of the models. However, the interaction between sex and *Spirulina* supplementation level was a significant source of variation in feed intake. These findings highlight the variation between commonly used feed intake models and permits Australian dual-purpose lamb producers to

manage and match feed resources with available sire breed, *Spirulina* supplementation level and sex.

(**Keywords**: *Arthrospira platensis*, *feed intake, Spirulina supplementation, feed use efficiency, sheep*)

**Introduction**

*Spirulina* (*Arthrospira platensis*) is a protein-rich cyanobacterium which has emerged as a potential livestock feed and supplement (Holman and, Malau-Aduli, 2012b). The nutritional quality of *Spirulina* suits Australian dual-purpose lamb production wherein supplements are routinely used to promote faster growth and heavier liveweights. Generally, oil seed meal and grains are used by these dual-purpose lamb producers because they are readily available and comparatively cheap (ABARE, 2012). Nonetheless, obtaining these supplements still contributes a major cost to lamb producers. The prices of feed supplements are influenced by drought due to climate change impacts in key production regions (Nardone et al., 2010), heightened competition for supplements as biofuels (Hegarty, 2012) and increased competition for land resources due to urban sprawl (Godfray et al., 2010). Therefore, the search for more cost effective supplements and the prudent use of feed resources and livestock that best match production systems is a focal point of on-going lamb production research.

Reduction of lamb feed intake without compromising growth would contribute to improved economic returns to dual-purpose lamb producers. Lamb genetics, feed supplementation level and type have all been previously found to affect voluntary feed intake requirements (Moore et al., 1999a). For instance, Doyle *et al.* (1988) found that the voluntary intake of oaten hay in lambs was depressed with higher rates of oat grain and sunflower meal supplementation. In contrast, Salisbury *et al.* (2004) reported an increase in forage intake with protein supplementation in wether lambs. Several models are available which incorporate liveweight and productivity parameters to provide adjusted feed intake information (Knott et al., 2008). To our

knowledge, there is a dearth of published literature on *Spirulina* supplementation and its impact on voluntary feed intake of genetically diverse meat sheep. Therefore, the major objective of this study was to investigate the effects of *Spirulina* supplementation level, sire breed and sex on the feed intake of dual-purpose Australian lambs using several feed intake models.

**Materials and Methods**

This study was conducted at the University of Tasmania Farm, Cambridge, Tasmania, Australia. All procedures had the University of Tasmania Animal Ethics approval and were conducted in accordance with the 1993 Tasmania Animal Welfare Act and the 2004 Australian code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004).

*Animal management and experimental design*

Purebred Merino ewes were mated with White Suffolk, Dorset and Merino terminal sires in separate paddocks to generate crossbred and purebred first filial generation progeny at the University of Tasmania Farm in Cambridge, Hobart, Australia. Lambs were identified using the Australian National Livestock Identification ear tags and weaned onto ryegrass pasture at 12 weeks of age. At 6 months of age, 24 lambs were randomly allocated into a balanced 3 x 4 x 2 completely randomised block feeding trial representing 3 sire breeds – Dorset, Merino, and White Suffolk; 4 *Spirulina* supplementation levels – CONTROL (0mL), LOW (50mL), MEDIUM (100mL) and HIGH (200mL); and 2 sexes – ewes and wethers.

*Spirulina* powder was purchased (TAAU, Darwin, NT, Australia) and dissolved into a water suspension using a *Spirulina* powder to water ratio of 1:10 w/v. Each lamb in the LOW, MEDIUM and HIGH *Spirulina* supplementation treatment group was orally drenched daily using a sheep drenching gun for the 9-week duration of the feeding trial that included a 3-week adjustment period. Throughout this study, all lambs were confined to individual 0.6 x 1.2 m metabolic crates with *ad libitum* access to

drinking water and the basal diet of Lucerne hay and 150 g of crushed barley grains, which were replaced daily. Prior to fresh servings, residual feeds were cleared from the feeding troughs and weighed to enable feed intake computation.

*Chemical analysis of feed components*

Dry matter content of the basal diets was determined by drying samples to a constant weight at 65°C in a fan forced oven. Ash content was determined by combusting samples in a furnace at 550°C for 5 hours. Neutral detergent fibre and acid detergent fibre contents were measured using an Ankom fibre analyser (ANKOM$^{220}$; ANKOM Technology, USA) (van Soest et al., 1991). Total N content was measured using the Kjeldahl method (van Soest et al., 1991) and the values were multiplied by 6.25 to give the crude protein percentage. Ether extract was determined using an Ankom fat/oil extractor (ANKOM$^{XT15}$; ANKOM Technology, USA) (ANKOM Technology, 2009). Metabolisable energy (ME) was calculated (Noblet and, Perez, 1993).

**Table 7.1** Chemical composition of feed components [1]

| | Feed components | | | Unit |
|---|---|---|---|---|
| | *Spirulina* | Barley grain | Lucerne hay | |
| DM | 96.0 | 93.2 | 90.6 | g/100g Fresh Wt |
| NDF | 32.6 | 18.5 | 36.0 | % DM |
| NDFn[2] | 30.3 | 17.2 | 33.5 | % DM |
| ADF | 18.3 | 6.0 | 29.0 | % DM |
| NFC[3] | 7.9 | 68.7 | 35.2 | % DM |
| Ash | 9.5 | 3.2 | 6.9 | % DM |
| EE | 5.9 | 2.0 | 1.9 | % DM |
| CP | 62.2 | 8.9 | 22.5 | % DM |
| ME[4] | 1707.5 | 1723.7 | 1689.3 | kJ/100g DM |

[1] Dry matter (DM), neutral detergent fibre (NDF), nitrogen free NDF (NDFn), non fibrous carbohydrate (NFC), acid detergent fibre (ADF), ether extract (EE), indigestible organic matter (IOM), and metabolisable energy (ME)
[2] NDFn = NDF x 0.93 (Undersander and, Moore, 2002)
[3] NFC = 100 – (NDFn + CP + EE + Ash) (Undersander and, Moore, 2002)
[4] ME = 4194 – (9.2 x Ash) + (1.9 x CP) + (3.9 x EE) – (3.5 x NDF) (Noblet and, Perez, 1993)

*Feed intake models*

This study used six models to calculate feed use efficiency (Table 7.2), being; 1) Residual feed intake (RFI) model which adjusts feed intake with lamb growth rates (Koch *et al.*, 1963); 2) Residual liveweight gain (RLG) model wherein feed intake is adjusted with lamb liveweights (Koch et al., 1963); 3) Standardised daily feed intake (SDFI) model which normalises feed intake, nutritional value and feeding trial duration (Knott et al., 2008); 4) Daily feed intake (DFI) which adjusts total feed intake to feeding trial duration; 5) Feed conversion ratio (FCR) wherein feed intake is adjusted to lamb liveweight (Refstie *et al.*, 1998); and 6) Metabolic feed conversion ratio ($FCR_{met}$) which adjusts lamb liveweight according to the total metabolic energy of feed ingested. Specific growth rate (SGR) was also calculated (Table 7.2).

**Table 7.2** Feed intake models and specific growth rate equations[1]

| Model | Equation | Abbreviations |
|---|---|---|
| RFI | $RFI = \beta_0 + \beta_1 \mathrm{ADG}_i + \beta_2 \mathrm{MWT}_i + e_i$ | $\beta_0$ = regression intercept, $\beta_1$ = partial regression coefficient of feed intake on…, $\beta_2$ = partial regression coefficient of feed intake on MWT (initial wt + half total gain), $e_i$ = residual error in feed intake of lamb, $totalFI$ = total feed intake over trial, $days$ = duration of feeding trial (days), $RationDM$ = ration dry mass, $RationME$ = ration metabolic energy (kJ), $DailySupp$ = supplement per head per day (kg), $SuppDM$ = supplement dry mass, $SuppME$ = supplement metabolic energy, $FI$ = total feed intake (kg), $LWTchange$ = change in liveweight over feeding trial (kg), $FI_{met}$ = total metabolic energy (ME) feed intake, $LWTchange$ = change in liveweight over feeding trial (kg) |
| RLG | $RLG = \beta_0 + \beta_2 \mathrm{MWT}_i + \beta_3 \mathrm{FI}_i + e_i$ | |
| SDFI | $SDFI = \left(\frac{totalFI}{days}\right)\left(\frac{RationDM \times RationME}{1000}\right) + DailySupp\left(\frac{SuppDM \times SuppME}{1000}\right)$ | |
| DFI | $DFI = \frac{totalFI}{days}$ | |
| FCR | $FCR = FI \times LWTchange^{-1}$ | |
| $FCR_{met}$ | $FCR_{met} = FI_{met} \times LWTchange^{-1}$ | |
| SGR | $SGR = 100 \times (LWTchange) \times days^{-1}$ | |

[1] Daily feed intake (DFI), residual feed intake model (RFI), residual liveweight gain model (RLG), standardised daily feed intake model (SDFI), feed conversion ratio (FCR), metabolic FCR ($FCR_{met}$), and specific growth rate (SGR)

*Statistical analysis*

All data were analysed using 'Statistical Analysis System' software (SAS Institute., 2009). Initially, summary statistics were computed with means, standard errors, standard deviations, minimum and maximum values scrutinised for errors and outliers. General linear models procedure (PROC GLM) in SAS (SAS Institute., 2009) was utilised in running a factorial analysis of variance by fitting an analytical model with the fixed effects of *Spirulina* supplementation level (CONTROL, LOW, MEDIUM, HIGH), sire breed (Dorset, Merino, White Suffolk), sex (ewes, wethers) and their second-order interactions and feed intake models (DFI, RFI, RLG, SDFI, FCR, $FCR_{met}$, SGR) as dependent variables. Duncan's multiple range test was used for mean separation at $p<0.05$ level of significance. Relationships between the various feed intake models were computed using Pearsons correlation coefficients (PROC CORR) in SAS (SAS Institute., 2009) and the level of significance was established using Bonferroni's probability pairwise comparison test at $P<0.05$ level of significance.

**Results**

Chemical composition of the *Spirulina* supplement and the basal diet of crushed barley grain and lucerne hay are presented in Table 7.1. It was apparent that *Spirulina* was almost three times more protein-dense (62.2 g/100gDM) than the combined basal diet. However, in terms of metabolisable energy, the supplement and basal diets were almost iso-energetic ranging from 1689-1723 kJ/100g DM. There was also sufficient dry matter (90-96 g/100g fresh weight) and crude fibre to meet the gut-fill needs of the lambs.

Regardless of the model investigated, the impact of *Spirulina* supplementation level on feed intake was not significant ($P>0.05$; Table 7.3). Likewise, sex had no independent effect on feed intake ($P>0.05$; Table 7.4). In contrast, sire breed significantly influenced feed intake utilising all models except FCR and $FCR_{met}$ ($P<0.05$; Table 7.4) with Merino-sired lambs having comparatively lower DFI, RLG and SDFI

and higher RFI than the other sire breeds. Feed intakes in lambs sired by Dorset and White Suffolk breeds were very similar regardless of the model utilised (Table 7.4).

**Table 7.3** Feed intake models least square means and standard error (LSM ± SE) of *Spirulina* supplementation level (S) and its interactions with sire breed (B) and sex (G) level of significance (*P* values) [1]

| | ***Spirulina* supplementation level** (S) | | | | *P* values[2] | | |
|---|---|---|---|---|---|---|---|
| | CONTROL (*n*6) | LOW (*n*6) | MEDIUM (*n*6) | HIGH (*n*6) | S | S x B | S x G |
| Model | | | | | | | |
| DFI | 1.33 ± 0.04 | 1.37 ± 0.05 | 1.34 ± 0.05 | 1.34 ± 0.05 | 0.270 | 0.341 | 0.078 |
| RFI | 66.76 ± 0.90 | 63.92 ± 2.50 | 65.82 ± 0.88 | 66.47 ± 2.16 | 0.467 | 0.339 | 0.645 |
| RLG | 19.14 ± 0.84 | 18.83 ± 0.68 | 18.44 ± 0.84 | 19.00 ± 0.60 | 0.802 | 0.651 | 0.874 |
| SDFI | 23.97 ± 0.67 | 24.90 ± 0.78 | 24.57 ± 0.79 | 25.08 ± 0.83 | 0.067 | 0.341 | 0.078 |
| FCR | 31.41 ± 4.54 | 28.75 ± 4.07 | 17.02 ± 1.70 | 16.39 ± 8.36 | 0.297 | 0.749 | 0.518 |
| $FCR_{met}$ | 6241.52 ± 919.99 | 5712.49 ± 788.16 | 3447.25 ± 357.12 | 3373.32 ± 1710.21 | 0.337 | 0.761 | 0.516 |
| SGR | 4.93 ± 1.00 | 5.10 ± 0.46 | 8.33 ± 1.03 | 4.59 ± 2.77 | 0.136 | 0.039 | 0.431 |

[1] Daily feed intake (DFI), residual feed intake model (RFI), residual liveweight gain model (RLG), standardised daily feed intake model (SDFI), feed conversion ratio (FCR), metabolic FCR ($FCR_{met}$), and specific growth rate (SGR).
[2] *Spirulina* supplementation level (S), sire breed (B), and sex (G).

**Table 7.4** Feed intake models level of significance (*P* values), least square means and standard error (LSM ± SE) of sire breed (B), sex (G) and their interactions

| | Sire breed (B) | | | Sex (G) | | *P* values[2] | | |
|---|---|---|---|---|---|---|---|---|
| | Dorset (*n*8) | Merino (*n*8) | White Suffolk (*n*8) | Ewe (*n*12) | Wether (*n*12) | B | G | B x G |
| Feed intake model | | | | | | | | |
| DFI | 1.41 ± 0.01$^{A}$ | 1.20 ± 0.02$^{B}$ | 1.42 ± 0.02$^{A}$ | 1.34 ± 0.03 | 1.35 ± 0.04 | 0.001 | 0.819 | 0.023 |
| RFI | 63.66 ± 0.63$^{B}$ | 69.06 ± 1.40$^{A}$ | 64.50 ± 1.57$^{B}$ | 64.36 ± 1.16 | 67.12 ± 1.17 | 0.030 | 0.078 | 0.315 |
| RLG | 19.38 ± 0.42$^{A}$ | 17.25 ± 0.33$^{B}$ | 19.93 ± 0.60$^{A}$ | 18.29 ± 0.41 | 19.42 ± 0.54 | 0.013 | 0.076 | 0.142 |
| SDFI | 25.63 ± 0.15$^{A}$ | 22.46 ± 0.34$^{B}$ | 25.80 ± 0.44$^{A}$ | 24.60 ± 0.41 | 24.66 ± 0.64 | 0.001 | 0.819 | 0.023 |
| FCR | 21.37 ± 7.20 | 23.13 ± 3.78 | 25.66 ± 3.33 | 23.19 ± 4.90 | 23.59 ± 3.04 | 0.858 | 0.951 | 0.467 |
| $FCR_{met}$ | 4252.28 ± 1452.67 | 4716.30 ± 744.24 | 5112.36 ± 652.26 | 4639.83 ± 978.53 | 4747.46 ± 612.50 | 0.863 | 0.936 | 0.464 |
| SGR | 4.85 ± 2.16 | 6.12 ± 0.86 | 6.25 ± 0.85 | 4.76 ± 1.36 | 6.72 ± 0.81 | 0.524 | 0.113 | 0.427 |

[1] Daily feed intake (DFI), residual feed intake model (RFI), residual liveweight gain model (RLG), standardised daily feed intake model (SDFI), feed conversion ratio (FCR), metabolic FCR ($FCR_{met}$), and specific growth rate (SGR).
[2] *Spirulina* supplementation level (S), sire breed (B), and sex (G).

Figure 7.1 indicated that there were significant interactions (P<0.039) between *Spirulina* supplementation level and sire breed on SGR where purebred Merino lambs in the HIGH level treatment group had the highest SGR, while crossbred lambs sired by Dorset and White Suffolk supplemented at the MEDIUM *Spirulina* level had higher SGR than unsupplemented lambs in the CONTROL treatment. Similarly, significant sire breed and sex interactions were found as depicted in Figure 7.2 in which purebred Merino ewes had higher DFI (*P*<0.023) and SDFI (*P*<0.023) than wethers. All other second-order interactions were not significant (*P*>0.05; Supplementary Articles).

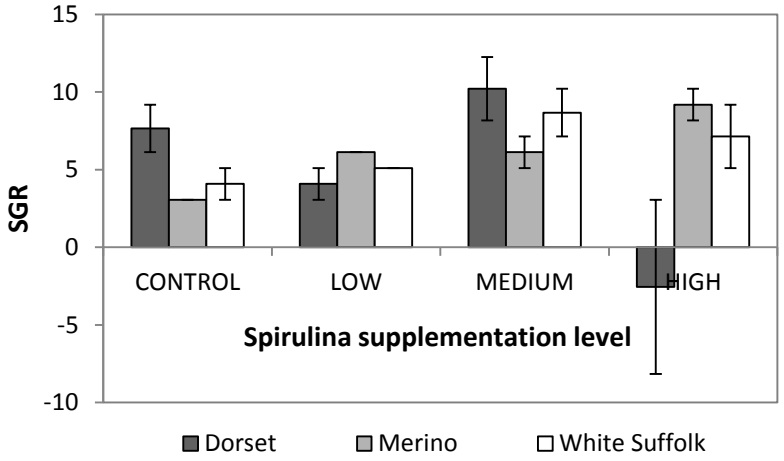


**Figure 7.10** *Spirulina* supplementation level and sire breed interactions on specific growth rate (SGR).

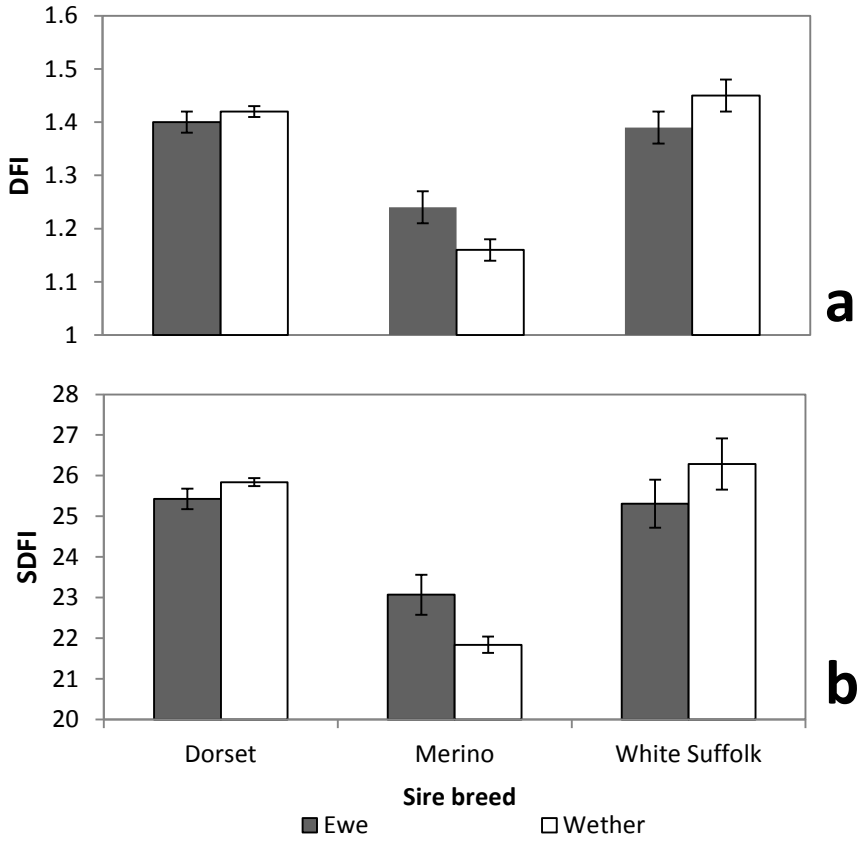


**Figure 7.11** Sire breed and sex interactions on: a) daily feed intake (DFI); and b) standardised daily feed intake (SDFI).

Relationships between all the feed intake models are portrayed in Table 7.5 where DFI was positively correlated with RLG and SDFI, but negatively correlated with RFI. FCR was strongly positively correlated with $FCR_{met}$ (0.999) as the latter was derived from the former. All other correlations were not significant (*P*>0.05).

**Table 7.5** Feed intake models Pearson's correlation coefficients [1]

| | DFI | FCR | SGR | RFI | RLG | SDFI |
|---|---|---|---|---|---|---|
| FCR | $0.039^{ns}$ | | | | | |
| SGR | $0.038^{ns}$ | $0.004^{ns}$ | | | | |
| RFI | -0.688*** | $-0.081^{ns}$ | $0.226^{ns}$ | | | |
| RLG | 0.695*** | $0.046^{ns}$ | $0.097^{ns}$ | $0.023^{ns}$ | | |
| SDFI | 0.977*** | $-0.0559^{ns}$ | $0.035^{ns}$ | -0.665*** | 0.679*** | |
| $FCR_{met}$ | $0.018^{ns}$ | 0.999*** | $0.012^{ns}$ | $-0.066^{ns}$ | $0.030^{ns}$ | $-0.072^{ns}$ |

[1] Daily feed intake (DFI), residual feed intake model (RFI), residual liveweight gain model (RLG), standardised daily feed intake model (SDFI), feed conversion ratio (FCR), metabolic FCR ($FCR_{met}$), and specific growth rate (SGR).

**Discussion**

Previous studies on the use of conventional protein-rich supplements such as canola meal in sheep had sought to improve the voluntary feed intake of grass-based diets and found that intake increased with increasing levels of canola meal supplementation (Hentz et al. 2012). Similar feed intake increases in steers fed low quality forages were also reported with increasing levels of rumen-degradable protein supplements (Bohnert et al. (2002). However, there have been other studies that reported lower feed intakes associated with supplementation in sheep (Salisbury et al. 2004; Ponnampalam *et al.* 2002; Lamb and Eadie 1979) and heifers (Loy et al. 2007). These varied outcomes in terms of the relationship between feed intake and protein supplementation in lambs are further exacerbated by the studies of Doyle et al. (1988) and Swanson et al. (2000) who reported that feed intake was not in any way affected by supplementation.

Oral drenching of sheep with *Spirulina* as a protein-rich supplement in Australian prime lamb production is an innovative and novel approach in pasture-based livestock systems. This study has demonstrated for the first time, that regardless of the level of supplementation, *Spirulina* did not affect feed intake in prime lambs irrespective of the model used. The implication is that the protein-dense composition of *Spirulina* has no detrimental effect on feed intake even if lambs are supplemented at LOW, MEDIUM or HIGH levels. It is pertinent to note that the effect of protein-rich supplementation on voluntary feed intake relies on protein content (Hentz et al., 2012) and the ratio of NDF to crude protein in a basal diet (Moore et al., 1999a). Feed intake has been shown to increase with protein-rich supplementation when NDF is relatively low, below approximately 1.2% liveweight, and *vice versa* (Ferrell et al., 1999). Hence, feed intake generally increases and decreases with supplementation when basal diets are of low- and high-quality respectively (Moore et al., 1999a; Salisbury et al., 2004). Therefore, being protein-rich, *Spirulina* had a minimal impact on total NDF intake.

Unlike sex, sire breed variation in mature size, growth and liveweight (Fogarty et al., 2005a; Hopkins et al., 2007a) significantly interacted with different levels of *Spirulina* supplementation to influence feed intake. Purebred Merino lambs are generally smaller than crossbreds at the same age, partly due to paternal genetic contributions and heterosis (Holman et al., 2012). Greater liveweight in the crossbreds is generally associated with greater feed intake to meet higher maintenance requirements (Hill, 2012). Comparatively greater feed use efficiencies in White Suffolk and Dorset specialist meat-type breeds than in purebred Merino lambs have been reported (Lee *et al.*, 1995; Mason, 1996). Our study herein conclusively demonstrates that prime lamb producers using *Spirulina* supplementation via oral drenching have accessible options of which sire breeds to use to match their production goal of higher feed intake and specific growth in lambs.

Feed intake models generally adjust feed intake data by various parameters associated with productivity such as liveweight, growth rates, feed nutritional value, and supplementation level (Knott et al., 2008; Lewis and, Emmans, 2010). Therefore, variation between models using the same information would be expected, unless models share a majority of parameters (Knott et al., 2008), as DFI and SDFI do within this study. The lack of widespread correlation between models observed highlights the diversity of parameters which contribute to accurate modelling of feed intake.

**Conclusion**

In conclusion, *Spirulina* supplementation level was shown not to affect modelled feed intake in purebred and crossbred Merino lambs. Sire breed affected feed intake with crossbred lambs SDFI and DFI exceeding purebred Merino. Similarly, purebred Merino ewes SDFI and DFI were comparatively higher than wethers. And, SGR was highest in crossbred Merino lambs supplemented MEDIUM *Spirulina* levels. These findings suggest *Spirulina* does not interact with voluntary feed intake as observed with other supplements due to its protein-rich and neutral detergent fibre-poor composition. Furthermore, the varied associations between models imply differing degrees of parameter duplication within their calculation. This highlights the scope

of parameters which contribute to accurate approximation of lamb feed intake. Essentially, this study provides allows Australian dual-purpose lamb producers to match and manage feed resources with *Spirulina* supplementation level, lamb sire breed and sex.

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Chapter 8

# Intramuscular fat percentage and subcutaneous fat melting point responses to *Spirulina* supplementation in lambs fed on high or low planes of nutrition

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**ABSTRACT**

We tested the effect of *Spirulina* supplementation on intramuscular fat percentage (IMF) and dominance of saturated (SFA) or unsaturated fatty acids (UFA) in dual-purpose crossbred and purebred Merino lambs. Over consecutive years, a total of 48 lambs were randomly allotted feeding trials (typical and simulated-drought basal diets), balanced by sire breed (Black Suffolk, Dorset, Merino, White Suffolk), sex (ewes, wethers), and *Spirulina* supplementation level (control [no *Spirulina*], low, medium, and high *Spirulina* supplementation). Each feeding trial continued for 9-weeks, with typical basal diet lambs run as a single mob with *ad libitum* access to ryegrass pasture; and simulated-drought basal diet lambs confined in metabolic crates with *ad libitum* access to Lucerne hay. Post-slaughter, *Longissimus dorsi* muscle samples were taken and IMF and SFA/UFA content was determined, using solvent extraction and subcutaneous fat melting point (FMP) methods respectively. Data was tested in SAS using Factorial ANOVA analysis with *Spirulina* supplementation, basal diet, sire breed, sex and their second-order interactions fitted as fixed effects and IMF and FMP as dependent variables. *Spirulina* supplementation was found to have no independent effect on IMF, whereas SFA content was heightened with high *Spirulina* supplementation levels. IMF and SFA content were highest with simulated-drought basal diets. And, IMF decreased with

increased *Spirulina* supplementation level and typical basal diet. As a result, our initial hypothesis that *Spirulina* supplementation would increase IMF and UFA content was rejected. These findings allow farmers to better manage *Spirulina* supplementation with IMF dependent eating qualities and SFA/UFA consumer health concerns considered, in these investigated dual-purpose lamb types.

(**Keywords:** *Arthrospira platensis, protein supplementation, meat quality, fatty acid composition, dual-purpose*)

**Introduction**

Australian dual-purpose lamb production must cater to consumer demands for a healthier product of high eating quality. Consequently, much research has focused on producing a leaner meat cut with increased levels of unsaturated fats (UFA) and decreased saturated fats (SFA). Meats rich in UFA have been associated with increased palatability (Siebert *et al.*, 2000) and consumer health benefits; reducing the risk of cardiovascular disease (Woods and, Fearon, 2009). Focus on lean meat production may have negative implication on intramuscular fat percentage (IMF) which is closely aligned with tenderness and juiciness, both fundamental meat eating qualities (Hopkins et al., 2007c; McPhee *et al.*, 2008). Fortunately, UFA and IMF can both be manipulated via managing lambs' basal diet and supplementing feed intake.

*Spirulina* (*Arthrospira platensis*), is a cyanobacterium which has been trialled as a supplementary feed in many livestock and animal species (Holman and, Malau-Aduli, 2012b). Its nutrient-rich composition, including many essential fatty acids, amino acids and carotenoids (Table 8.1), makes it an ideal dual-purpose lamb supplement. Yet, to the best of our knowledge, the effect of *Spirulina* supplementation on UFA/SFA and IMF has only been trialled in rabbits (Peiretti and, Meineri, 2011). Therefore, our objective was to evaluate the effect of *Spirulina* supplementation and its interactions with sire breed, sex and basal diet on IMF and UFA/SFA in purebred and crossbred Merino lambs. We hypothesised significant incremental increases in IMF and UFA within lambs' supplemented increasing *Spirulina* levels.

**Materials and Methods**

This study was conducted at the University Farm, Cambridge TAS under provision of the University of Tasmania Animal Ethics committee being in accordance with the 1993 Tasmania Animal Welfare Act and the 2004 Australian code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004).

*Animal management and experimental design*

Over consecutive years (2011-12) 16 rams were mated with approximately 1600 purebred Merino ewes in a 1:100 mating ratio. Progeny were identified using National Livestock Identification ear tags and weaned onto ryegrass pastures at 12 weeks of age. At 6 months old, 24 lambs were randomly selected for each feeding trial; typical basal diet (2011), and simulated-drought basal diet (2012). Feeding trials continued over 9-weeks.

*Spirulina* powder was purchased (TAAU, Darwin, AUS) and made into a water suspension using a 1:10 w/v ratio – *Spirulina* (g): water (ml). Each lamb was directly provided its assigned *Spirulina* supplementation level daily using a sheep drench.

*Typical basal diet:* Lamb selection was balanced by *Spirulina* supplementation level – control (0mL), medium *Spirulina* supplementation (100mL), and high *Spirulina* supplementation (200mL); sire breed – Black Suffolk, Dorset, Merino and White Suffolk; and sex – ewes and wethers. All lambs were run as a single block with ad libitum access to drinking water and a basal diet of ryegrass pasture and barley grain (Table 8.1).

*Simulated-drought basal diet:* Lambs' were assigned treatments balanced by *Spirulina* supplementation levels – control (0mL), low *Spirulina* supplementation (50mL), medium *Spirulina* supplementation (100mL), and high *Spirulina* supplementation (200mL); sire breed – Dorset, Merino and White Suffolk; and sex –

ewes and wethers. Lambs' were maintained in individual 0.6m x 1.2m metabolic crates with *ad libitum* access to drinking water and Lucerne hay, which was replaced daily. All lambs received cracked barley (150g/day).

**Table 8.14** Chemical composition of *Spirulina*, a summary [1, 2]

| | Amount | Unit |
|---|---|---|
| Moisture | 4 – 9 | %DM |
| Fat (Mojonnier extraction) | 4 – 16 | %DM |
| Protein (N x 6.25) | 60 – 70 | %DM |
| Ash | 3 – 11 | %DM |
| Carbohydrates (total) | 14 – 19 | %DM |
| Energy | 1504 – 1708 | kJ/100g DM |
| Crude fibre | 3 – 7 | %DM |
| | | |
| Total carotenoids | 1700 | mg/kg |
| Beta-carotene | 140000 | µg/100g |
| | | |
| 16:0 | 25.8 – 44.9 | % total FA |
| 16:1ω6 | 2.3 – 3.8 | % total FA |
| 18:0 | 1.7 – 2.2 | % total FA |
| 18:1ω6 | 10.1 – 16.6 | % total FA |
| 18:2ω6 | 11.1 – 12.0 | % total FA |
| 18:3ω6 | 17.1 – 40.1 | % total FA |

[1] Dry matter (DM), fatty acids (FA)
[2] Adapted from (Holman and, Malau-Aduli, 2012b)

*Slaughter and sample preparation*

Slaughter occurred at a commercial abattoir (Gretna Quality Meats, Gretna, AUS) on June 9 2011 and May 24 2012. On both occasions, *Longissimus dorsi* muscle samples with overlaying subcutaneous fat were removed from each carcass. These were catalogued and stored at -20°C until analysis. Prior to analysis, samples were thawed for 24 hours under refrigeration.

*Determination of intramuscular fat percentage*

IMF was found using a modified Folch *et al*. (1957) protocol. This involved using a Ronson homogeniser to homogenise 10g of subcutaneous fat free tissue from each sample. In triplicate, approximately 1g of this was then shaken vigorously for 5 minutes with 5.5mL of choloform:methanol (2:1 vol/vol) solvent. The solution was filtered and combined with a secondary solvent extraction on the sample tissue sample. These filtrate solutions were added to 5.5mL potassium chloride (1.34mol/L$^{-}$

[1]) and allowed to precipitate into two layers. The lower layer was transferred to a pre-weighed and labelled ceramic crucible before being evaporated on a heating block for 1 hour, cooled and placed in a desiccator to dry. Upon weight stabilisation, the weight of intramuscular fat was calculated as Final wt – Initial crucible wt. This was converted to IMF by: wt(g)/initial tissue subsample(g)*100.

*Determination of subcutaneous fat melting point*

Subcutaneous fat was removed from each sample and melted in a labelled ceramic crucible in an oven at 100°C for 30 minutes. Molten lipid was transferred into 100mm Melting Point Determining Tubes (Both ends opened; Hirshmann Laborgerate®) with samples represented in triplicates. These were refrigerated prior to FMP evaluation using AOCS (2009) methodology, wherein the temperature at 'slip point' was recorded.

*Statistical analysis*

Data was analysed using 'Statistical Analysis System' software (SAS Institute., 2009). Summary statistics were computed with means, standard deviations, and minimum and maximum values examined for data entry errors and outliers. Factorial ANOVA (PROC GLM) analysis (SAS Institute., 2009) was run with *Spirulina* supplementation, basal diet, sire breed, sex and their second-order interactions fitted as fixed effects and IMF and FMP as dependent variables. Duncan's multiple range and Bonferroni's probability pairwise comparison tests were used to distinguish differing means ($P<0.05$).

**Results**

*Intramuscular fat percentage and subcutaneous fat melting point independent effects*

IMF was highest with low *Spirulina* supplementation levels and typical basal diet. Neither sire breed nor sex independently effected IMF. Low and high *Spirulina*

supplementation level FMP exceeded that found for control and medium *Spirulina* supplementation levels. Merino-sired lambs FMP was highest, followed sequentially by Dorset-, White Suffolk- and Black Suffolk-sired lambs. Simulated-drought basal diet FMP was highest. No other effects were significant (*P*>0.05; Table 8.2).

**Table 8.15** *Spirulina* supplementation level, sire breed, sex and plane of nutrition independent effects on intramuscular fat percentage and subcutaneous fat melting point [1, 2, 3]

| | | IMF (%) | FMP (°C) |
|---|---|---|---|
| ***Spirulina* level** | CONTROL | 2.29 ± 0.22$^{B}$ | 43.17 ± 0.45$^{B}$ |
| | LOW | 3.18 ± 0.43$^{A}$ | 44.44 ± 0.45$^{A}$ |
| | MEDIUM | 1.98 ± 0.13$^{B}$ | 42.97 ± 0.45$^{B}$ |
| | HIGH | 2.33 ± 0.28$^{B}$ | 44.86 ± 0.19$^{A}$ |
| | *P values* | ** | *** |
| **Sire breed** | Black Suffolk | 1.59 ± 0.12 | 41.53 ± 0.60$^{D}$ |
| | Dorset | 2.38 ± 0.21 | 44.21 ± 0.32$^{B}$ |
| | Merino | 2.22 ± 0.34 | 45.63 ± 0.33$^{A}$ |
| | White Suffolk | 2.52 ± 0.22 | 43.25 ± 0.30C |
| | *P values* | $^{NS}$ | *** |
| **Sex** | Ewe | 2.52 ± 0.19 | 43.59 ± 0.33 |
| | Wether | 2.12 ± 0.16 | 43.86 ± 0.26 |
| | *P values* | * | $^{NS}$ |
| **Plane of nutrition** | High | 1.65 ± 0.11$^{B}$ | 43.67 ± 0.31$^{B}$ |
| | Low | 2.93 ± 0.19$^{A}$ | 43.81 ± 0.28$^{A}$ |
| | *P values* | *** | *** |

[1] Means within fixed effect columns with different superscripts significantly differ (*P*<0.05)
[2] Level of significant: $^{NS}$ no significance (*P*>0.05), * significant (*P*<0.05), ** highly significant (P<0.01), *** very highly significant (*P*<0.001)
[3] Intramuscular fat percentage (IMF), subcutaneous fat melting point (FMP)

*Intramuscular fat percentage and subcutaneous fat melting point second-order interaction effects*

IMF was lowest with high *Spirulina* supplementation levels and typical basal diet. IMF was highest with high *Spirulina* supplementation levels and simulated-drought basal diet (Fig. 8.1a). Simulated-drought basal diet FMP was highest with high and low *Spirulina* supplementation levels (Fig. 8.1b). No other second-order interaction effect were significant (*P*>0.05).

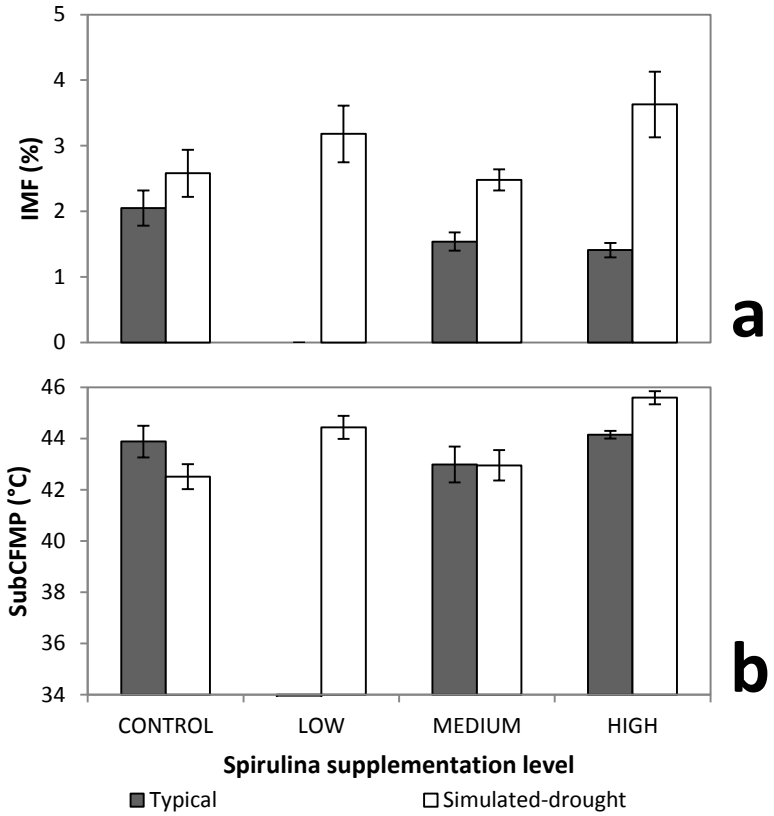


**Figure 8.12** *Spirulina* supplementation level and plane of nutrition interactions on: a) Intramuscular fat percentage (IMF; *P*<0.001); and b) subcutaneous fat melting point (FMP; *P*<0.001).

*Intramuscular fat percentage and subcutaneous fat melting point third-order interaction effects*

Ewe IMF with typical basal diet and medium and high *Spirulina* supplementation levels were higher than control. Ewe IMF with high *Spirulina* supplementation level and simulated-drought basal diet exceeded all other IMF findings (Fig. 8.2a). Ewe FMP with medium *Spirulina* supplementation levels and typical basal diet was lowest. Ewe FMP was highest with medium and high *Spirulina* supplementation and simulated-drought basal diet. Wether FMP with low and high *Spirulina* supplementation and simulated-drought basal diet exceeded control and medium *Spirulina* supplementation levels (Fig. 8.2b).

Black Suffolk-sired lambs' FMP was highest with high *Spirulina* supplementation and typical basal diet. Merino-sired lambs' FMP was highest with medium *Spirulina*

supplementation and typical basal diet. Dorset-sired lambs' FMP was higher with *Spirulina* supplementation and simulated-drought conditions comparative to control. White Suffolk-sired lambs FMP was highest with high *Spirulina* supplementation and simulated-drought conditions (Fig. 8.3). No other third-order interactions were significant ($P$>0.05).

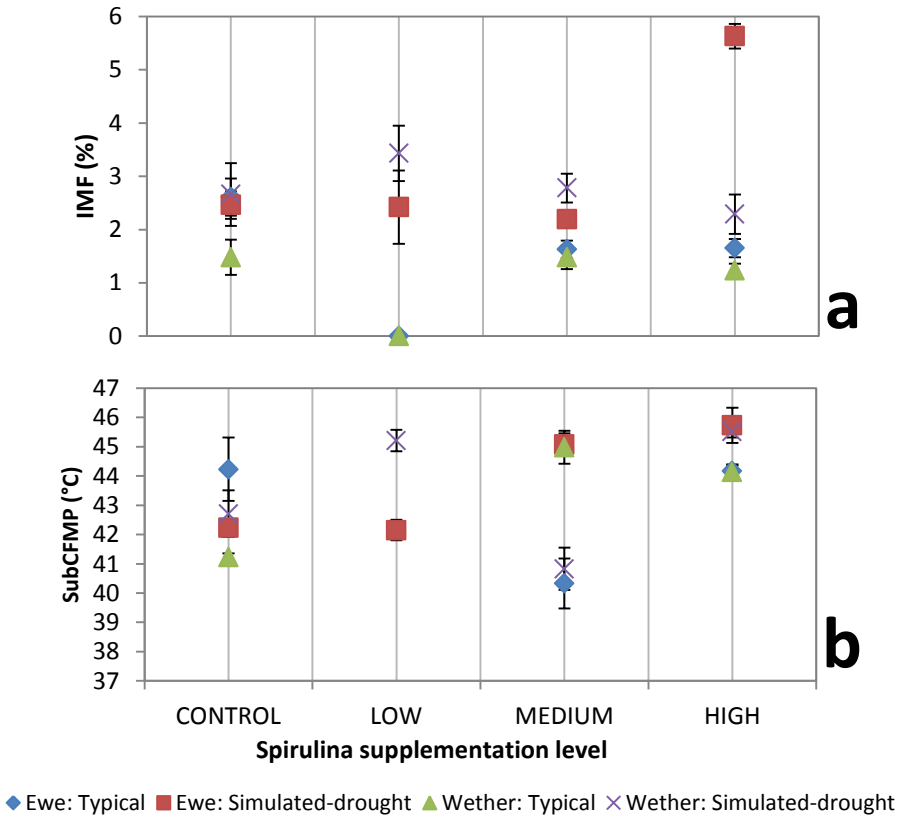


**Figure 8.13** *Spirulina* supplementation level, sex and plane of nutrition interactions on: a) Intramuscular fat percentage (IMF; $P$<0.001); and b) subcutaneous fat melting point (FMP; $P$<0.001).

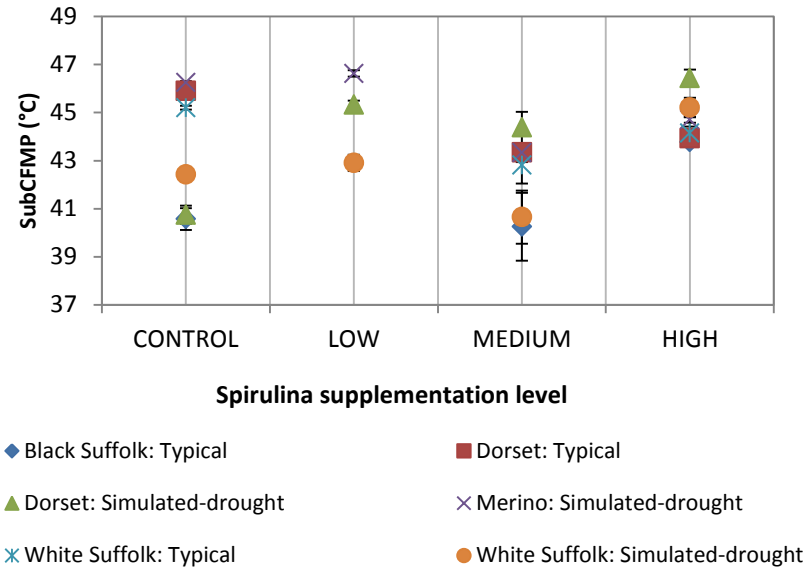


**Figure 8.14** *Spirulina* supplementation level, sex and sire breed interactions on subcutaneous fat melting point (FMP; *P*<0.001).

## Discussion

*Intramuscular fat percentage was not influenced by Spirulina supplementation*

Observed IMF with low *Spirulina* supplementation levels' significant difference is thought to only have occurred due to its exclusion during the typical basal diet feeding trial. Therefore, it is not comparable to other *Spirulina* supplementation levels as an independent variable. Furthermore, with no other IMF differences observed, we can assume no difference in lamb eating qualities associated with IMF exist as a result of *Spirulina* supplementation.

*Subcutaneous fat melting point was highest with lambs supplemented HIGH Spirulina levels*

For the previously stated reasons, low *Spirulina* supplementation level FMP can also be disregarded. Consequently, FMP was shown to be heightened with *Spirulina* supplementation, suggesting an increase in SFA and decrease in UFA within subcutaneous fat of these lambs. This outcome is thought to have arisen from *Spirulina*'s naturally high β-carotene content (Table 1; Holman and, Malau-Aduli,

2012b). Retinoic acid, a metabolite of this β-carotene, interferes with ruminant stearoyl Co-A desaturase expression and limits SFA desaturation in the rumen (Siebert et al., 2000). Hence, rich β-carotene rations, including high *Spirulina* supplementation levels, would cause a lower desaturation of SFA and higher FMP comparative to poor β-carotene rations (Siebert et al., 2000). These changes in SFA/UFA differ from supplementary trials giving *Spirulina* to rabbits (Peiretti and, Meineri, 2011). Yet, rabbits are pseudo-ruminants unlike ruminant lambs wherein dietary *Spirulina*'s UFA is relatively unprotected from biohydrogensation by rumen microflora.

*Simulated-drought basal diets resulted in higher intramuscular fat percentages than typical basal diets*

Dietary intake has a marked effect on ruminant IMF; with low intake, such as those characteristic of drought, resulting in reduced IMF comparative to high intake diets (Geesink and, Zerby, 2010; Hopkins et al., 2007c; Roberts *et al.*, 2007). This study's findings, however, diverge from this preconception. This may have arisen from our experimental design in which simulated-drought lambs were confined to stalls. This confinement would have restricted energy expenditure to well below that of equivalent lambs with a typical basal diet and maintained on a pasture. We suggest this imbalance of energy utilisation would be reflected in our IMF findings.

*Simulated-drought basal diets resulted in higher subcutaneous fat melting points compared to lambs under typical basal diets*

High FMP are associated with elevated SFA and diminutive UFA concentrations. And, dietary nutritional factors influence ruminant adipose deposits' fatty acid composition (Webb and, O'Neill, 2008), although to a lesser extent than in monogastrics (Bas and, Morand-Fehr, 2000). Dietary fatty acids influence on adipose deposits' fatty acid composition is limited by rumen microflora biohydrogensation of UFA (Bas and, Morand-Fehr, 2000). However, linolenic acid, a majory proportion of pasture's fatty acid profile, is relatively resistant to this biohydrogensation compared to other fatty acids (Bas and, Morand-Fehr, 2000). Furthermore, dietary linolenic

acid has been reported to increase ruminant UFA levels, such as eicosapentaenoic acid, docosahexaenoic acid and docosapentaenoic acid (Raes et al., 2004). Subsequently, we can theorise that lambs fed the pasture-rich diet in typical basal diets would have greater UFA and lower FMP compared to those fed the pasture-lacking diet in simulated-drought conditions, as found. Aurousseau *et al.* (2007b) shared similar findings, with lambs finished on both pasture and concentrates UFA being lower than stall fed counterparts fed only concentrates.

*Intramuscular fat percentage decrease with rising Spirulina supplementation levels and typical diets was not observed with simulated-drought basal diet*

To reiterate, IMF is influenced by dietary nutrition, particularly lipid content. This premise would explain our found difference between medium and high *Spirulina* supplementation levels with simulated-drought basal diet. However, our findings with typical basal diets are unexplained. Instead, we must understand the tendency of dietary lipid to be partitioned more so to subcutaneous fat deposits than IMF (Kempster, 1981). Holman *et al*. (2012) reports of an incremental increase in lamb body condition score with increased *Spirulina* supplementation level in these same lambs. And, as body condition score is quintessentially an assessment of the degree of subcutaneous fat deposition. Hence, the opposing response of IMF and subcutaneous fat deposition to *Spirulina* supplementation allows us to conclude that dietary lipid sourced from *Spirulina* was partitioned primarily to the later depot. Therefore, *Spirulina* supplementation with typical basal diet results in leaner lamb meat compared to control lambs'.

*Sire breed and sex effected both intramuscular fat percentage and subcutaneous fat melting points*

Ruminant sire breed effects on IMF (McPhee et al., 2008; Pethick et al., 2010b) and FMP (Aurousseau et al., 2007b; Costa *et al.*, 2009; Malau-Aduli et al., 2000c) have been widely reviewed previously. As has ruminant sex's influence on IMF (Dervishi *et al.*, 2012; McPhee et al., 2008; Pethick et al., 2010b).

**Conclusion**

*Spirulina* supplementation was shown to not affect lamb IMF, unless through interactions with basal diet type. Typical basal diet resulted in incremental decline of IMF with increased *Spirulina* supplementation levels. Subcutaneous SFA, assessed using FMP, were increased with high *Spirulina* supplementation level. Lambs' with simulated-drought basal diets SFA was greater than counterparts with typical basal diet. These findings highlight *Spirulina*'s potential in altering meat quality and health properties when used as a dual-purpose lamb supplementary feed. They also prompt us to reject our initial hypothesis, that; *Spirulina* will incrementally increase IMF and UFA when supplemented at increasing levels to purebred and crossbred Merino lambs. It is thought that further research into *Spirulina* supplementation effect on lamb meat quality and effect on total fatty acid profiles would complement this study.

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*Chapter 9*

# Sire breed and sex variations in the fatty acid composition of heart, kidney, liver, adipose and muscle tissues of crossbred and purebred lambs

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**Abstract**

We investigated sire breed and sex effects on lamb subcutaneous adipose, *Longissimus dorsi* muscle, kidney, heart and liver tissues fatty acid (FA) composition. Tissues were sampled from 40 ewe and wether lambs sired by Black Suffolk, Dorset, Merino and White Suffolk. FA profiles obtained by gas chromatography were transformed to percentages of total FA and analysed. Total saturated FA were highest in adipose; monounsaturated FA (MUFA) in muscle; eicosapentaenoic and docosapentaenoic acids in kidney; and, total polyunsaturated FA (PUFA) in the heart. Sire breed affected kidney 18:3n-3, 22:6n-3, 16:0, 16:1n-7c and total n-6; muscle 17:0, 18:3n-3 and 18:1n-7; adipose 16:1n-9c; and, 17:0; and, 16:0 in the heart and liver tissue levels. Muscle tissue from wether lambs had highest 18:1n-9 levels. Therefore heart, liver and kidney consumption would be beneficial because of total PUFA. Sire breed variations imply that lamb products FA composition can be manipulated using breed management.

(**Keywords**: *Sheep, Fatty acid composition, Sire breed, Meat quality, Sex*)

**Introduction**

Achieving a higher quality of lamb product is fundamental to the economic success of dual-purpose and prime lamb operations. This can be focused towards improving the nutritional and sensory appeal of lamb products. Fortunately, both objectives are achievable through intrinsic sire breed selection and nutritional manipulation of fatty acid (FA) composition and management of key lamb tissues (Doreau et al., 2011; Raes et al., 2004; Wood *et al.*, 2004). Of particular relevance is the *Longissimus dorsi* muscle, subcutaneous adipose, kidney, heart and liver FA composition as these tissues are typically the consumables taken from a lamb carcass, albeit dependent strongly on cultural background.

FA composition influences lamb product sensory quality through flavour and appearance. For instance, lipid colour, flavour and shelf-life have all been shown to be elevated when FA profile is dominated by saturated FA (SFA) as these are better resistant to oxidation than unsaturated FA (UFA) (Wood et al., 2004). In contrast, lamb product nutritional quality is decreased with a SFA dominated FA composition and improved with increased UFA (Doreau et al., 2011), especially when polyunsaturated (PUFA). Moreover, nutritional quality is improved with increased levels of long chain omega-3 (n-3) FA (Fisher et al., 2000; Scollan et al., 2001), particularly eicosapentaenoic acid (20:5n-3; EPA), docosapentaenoic acid (22:5n-3; DPA), arachidonic acid (20:4n-3; ETA) and docosahexaenoic acid (22:6n-3; DHA) (Raes et al., 2004). This is as a result of the strong association of these FA with reductions in cardiovascular disease risk and the promotion of improved mental health and infant development with their consumption (Howe et al., 2006).

Dietary manipulation has been widely demonstrated as a viable means of managing the FA composition of lamb (Cooper *et al.*, 2004; Doreau et al., 2011; Fisher et al., 2000). Genetic manipulation of FA composition in lambs is an alternative, cumulative and a comparatively more permanent route than by nutritional means. Selection of sire breeds to produce progeny with desirable FA profiles has been a powerful means of effecting genetic changes. However, rate of progress will depend on how

genetically divergent the sire breeds are and the heritability of the trait. Fisher et al., (2000) reported Suffolk lambs as having lower PUFA and higher contents of palmitic and oleic acids within the *semimembranosus* muscle tissue than Soay lambs (Mezoszentgyorgyi et al., 2001). Likewise, higher SFA content was found in female lambs than males in subcutaneous adipose tissue (Mezoszentgyorgyi et al., 2001). Therefore, lamb product FA composition can theoretically be managed via genetic tools such as crossbreeding that maximize the utilization of sire breed and sex variations. However, to the best of our knowledge for the typical Australian dual-purpose and prime lamb pasture-based operations, the effect of sire breed and sex on the FA composition of key tissues and organs of purebred and crossbred Merino lambs has seen only very limited investigation. Furthermore, no comparison between FA compositions of these consumed lamb tissue and organs, or response to crossbreeding has been reported.

Therefore, the major objective of this study was to investigate the effects of sire breed and sex on the FA composition of subcutaneous adipose, *Longissimus dorsi* muscle, kidney, heart and liver tissues in dual-purpose Australian lambs. It was hypothesized that sire breed and sex would affect lamb tissue and organ FA composition, and independently, these tissues and organs would also differ in their FA profiles.

**Materials and Methods**

All experimental procedures were in accordance with the University of Tasmania (UTAS) Animal Ethics Committee guidelines, the 1993 Tasmania Animal Welfare Act and the 2004 Australian code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004). This study was conducted at the UTAS Farm, Cambridge TAS, Australia.

*Animal management, slaughter and sampling*

Over two consecutive years, Dorset, Merino, White Suffolk and Black Suffolk terminal sires were mated with purebred Merino ewes at the University of Tasmania Farm, Cambridge, using a 1:100 ram to ewe mating ratio. All resulting $F_1$ progeny were identified using National Livestock Identification ear tags and weaned onto ryegrass pastures at 12 weeks of age. Each year, at 6 months of age, a total of 40 weaner lambs was selected for a 9-week feeding trial on either a basal ryegrass pasture or simulated drought lucerne hay. All lambs were offered the same protein-rich supplementation routine described by Holman et al., (2012), with the basal diet or nutritional planes differing between years: Year 1) pasture-based diet or high nutritional plane; and Year 2) simulated-drought diet or low nutritional plane. Lamb selection and allocation into treatment groups was random, but balanced by sex (ewes, wethers) and sire breed: Year 1) Black Suffolk, Dorset, Merino, and White Suffolk; and Year 2) Dorset, Merino and White Suffolk.

At the completion of each feeding trial, all experimental lambs were slaughtered at a commercial abattoir (Gretna Quality Meats, Gretna, Tasmania, AUS), except the Merino-sired ewe lambs that were saved for breeding purposes. On both occasions, *Longissimus dorsi* muscle with overlaying subcutaneous adipose deposit, kidney, liver and heart tissue samples were immediately removed from each carcass, snap-frozen in liquid nitrogen, transported to the laboratory and stored at -20°C until analysis of fatty acids.

*Lipid extraction and analysis*

Subcutaneous adipose, kidney, heart, liver and muscle tissue samples were extracted overnight using a modified Bligh and Dyer protocol (Bligh and, Dyer, 1959). As an overview, a single-phase overnight extraction using $CHCl_3$:MeOH:$H_2O$ (1:2:0.8 v/v) followed by phase separation with $CHCl_3$:saline$H_2O$ (1:1 v/v) was used. The total lipid extract was obtained via rotary evaporation of the lower chloroform phase.

An aliquot from each samples total lipid extract was transmethylated in MeOH:$CHCl_3$:HCl (10:1:1 v/v) for 2h at 80°C. Milli-Q $H_2O$ (1ml) was then added before FA methyl esters (FAME) were extracted with hexane:chloroform (4:1 v/v) and reduced under a nitrogen stream and a known concentration added of an internal injection standard - 19:0 FAME. An Agilent Technologies 7890B gas chromatograph (GC) (Palo Alto, California USA) equipped with an Equity™-1 fused silica capillary column (15m x 0.1mm internal diameter and 0.1µm film thickness), a flame ionisation detector, a split/splitless injector and an Agilent Technologies 7683 B Series autosampler was used in analysis. Samples were injected in splitless mode, carried by helium gas, at an oven temperature of 120°C. Post-injection, oven temperature was raised to 270°C at 10°C/min, then to 310°C at 5°C/min. Peaks were quantified by Agilent Technologies ChemStation software (Palo Alto, California USA).

FA identities were confirmed using GC-mass spectrometric (GC/MS) analysis. These were performed using a Finnigan Thermoquest GCQ GC-MS fitted with an on-column injector and using Thermoquest Xcalibur software (Austin, Texas USA). The GC had a HP-5 cross-linked methyl silicone-fused silica capillary column (50m x 0.32mm internal diameter). The carrier gas used was helium, with operating conditions previously described (Miller *et al.*, 2006).

**Table 9.1** Basal diet components mean fatty acid composition (% total FA), content (mg/100g) and standard errors (±SEM)[1]

| | Content | | | | | | | | Composition | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pasture | | Hay | | Barley | | *Spirulina* | | Pasture | | Hay | | Barley | | *Spirulina* | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 11.1 | 6.4 | 0.4 | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 | 1.9 | 0.4 | 0.7 | 0.1 | 0.3 | 0.0 | 0.0 | 0.0 |
| 15:0 | 3.3 | 2.9 | 1.7 | 0.1 | 0.3 | 0.0 | 0.2 | 0.2 | 0.4 | 0.2 | 2.9 | 0.0 | 0.1 | 0.0 | 0.8 | 0.8 |
| 16:3 | 0.0 | 0.0 | 0.2 | 0.2 | 0.1 | 0.1 | . | . | 0.0 | 0.0 | 0.3 | 0.3 | 0.0 | 0.0 | . | . |
| 16:1n7c | 4.9 | 3.3 | 1.0 | 0.1 | 1.0 | 0.0 | 5.6 | 5.6 | 0.7 | 0.0 | 1.7 | 0.1 | 0.2 | 0.0 | 2.2 | 2.2 |
| 16:0 | 166.7 | 118.4 | 26.3 | 2.4 | 103.5 | 11.9 | 79.6 | 65.9 | 24.4 | 1.2 | 45.6 | 0.6 | 25.7 | 0.0 | 53.2 | 3.6 |
| 17:1n-8c | 3.1 | 2.1 | 0.4 | 0.0 | 0.2 | 0.0 | 0.4 | 0.4 | 0.5 | 0.0 | 0.7 | 0.1 | 0.0 | 0.0 | 0.2 | 0.2 |
| 17:0 | 1.3 | 0.3 | 0.8 | 0.0 | 0.4 | 0.0 | 0.4 | 0.4 | 0.4 | 0.3 | 1.4 | 0.0 | 0.1 | 0.0 | 0.2 | 0.2 |
| 18:2n-6 | 44.2 | 31.1 | 7.4 | 0.6 | 196.7 | 29.0 | 20.5 | 20.5 | 6.5 | 0.2 | 12.8 | 0.0 | 48.6 | 1.6 | 8.0 | 8.0 |
| 18:3n-3 ALA | 272.1 | 185.9 | 3.2 | 0.4 | 22.1 | 2.2 | 1.0 | 1.0 | 41.3 | 0.2 | 5.6 | 0.2 | 5.6 | 1.2 | 3.6 | 3.6 |
| 18:1n-9c | 81.0 | 55.9 | 3.5 | 0.4 | 52.0 | 6.4 | 6.9 | 0.1 | 12.2 | 0.1 | 6.1 | 0.2 | 12.9 | 0.1 | 13.7 | 11.0 |
| 18:1n-7c | 6.0 | 4.2 | 0.8 | 0.1 | 4.1 | 0.6 | 0.8 | 0.8 | 0.9 | 0.0 | 1.5 | 0.1 | 1.0 | 0.0 | 0.3 | 0.3 |
| 18:0 | 45.1 | 30.4 | 4.5 | 0.3 | 6.3 | 0.0 | 2.5 | 2.1 | 6.9 | 0.1 | 7.9 | 0.1 | 1.6 | 0.2 | 1.6 | 0.3 |
| 20:5n-3 | 0.6 | 0.6 | 0.1 | 0.1 | 0.6 | 0.2 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.0 | 0.0 | 0.0 |
| 20:3n-6 | 0.2 | 0.2 | 0.2 | 0.0 | 1.7 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.0 | 0.4 | 0.0 | 0.0 | 0.0 |
| 20:4n-3 | 0.0 | 0.0 | 0.5 | 0.1 | 3.2 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.9 | 0.1 | 0.8 | 0.1 | 0.0 | 0.0 |
| 20:2n-6 | 4.9 | 3.0 | 0.0 | 0.0 | 0.5 | 0.2 | 0.0 | 0.0 | 0.8 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 |
| 20:0 | 3.1 | 1.7 | 1.2 | 0.1 | 0.8 | 0.1 | 0.0 | 0.0 | 0.5 | 0.1 | 2.1 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| 22:5n-6 | 0.5 | 0.0 | 0.0 | 0.0 | 1.1 | 0.5 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.3 | 0.2 | 0.0 | 0.0 |
| 22:4n-6 | 0.5 | 0.5 | 0.0 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| 22:5n-3 | 1.0 | 0.4 | 0.0 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 | 0.4 | 0.3 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| 22:1n-11c | 1.0 | 1.0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:1n-9c | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.1 | . | . | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | . | . |
| 22:0 | 2.2 | 0.8 | 1.8 | 0.0 | 1.2 | 0.3 | 0.0 | 0.0 | 0.5 | 0.2 | 3.2 | 0.2 | 0.3 | 0.1 | 0.0 | 0.0 |
| 24:6n-3 | 0.1 | 0.1 | 1.0 | 0.0 | 0.6 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 0.2 | 0.1 | 0.0 | 0.0 | 0.0 |
| 24:0 | 1.0 | 0.3 | 1.8 | 0.0 | 0.6 | 0.1 | 0.0 | 0.0 | 0.2 | 0.1 | 3.2 | 0.3 | 0.2 | 0.0 | 0.0 | 0.0 |
| ∑SFA | 233.9 | 160.7 | 38.5 | 2.9 | 114.5 | 11.7 | 82.7 | 68.3 | 35.3 | 0.1 | 67.2 | 0.2 | 28.4 | 0.4 | 55.6 | 3.3 |
| ∑MUFA | 403.0 | 120.4 | 256.5 | 21.8 | 313.3 | 10.3 | 16.8 | 6.7 | 15.1 | 0.2 | 10.5 | 0.3 | 15.0 | 0.1 | 23.0 | 13.8 |
| ∑PUFA | 326.0 | 222.4 | 12.8 | 1.0 | 228.0 | 27.6 | 42.3 | 39.3 | 49.6 | 0.2 | 22.3 | 0.1 | 56.5 | 0.3 | 21.5 | 10.5 |
| ∑n-3 | 275.6 | 187.5 | 5.0 | 0.2 | 27.2 | 1.2 | 2.5 | 0.2 | 41.9 | 0.3 | 8.4 | 0.0 | 6.9 | 1.1 | 5.1 | 4.2 |
| ∑n-6 | 50.3 | 34.8 | 7.6 | 0.6 | 200.7 | 28.7 | 39.7 | 39.2 | 7.6 | 0.1 | 13.4 | 0.2 | 49.6 | 1.4 | 16.3 | 14.6 |
| ∑LCn-3 | 2.7 | 0.8 | 1.6 | 0.0 | 5.1 | 1.0 | 0.0 | 0.0 | 0.5 | 0.2 | 2.8 | 0.2 | 1.2 | 0.1 | 0.0 | 0.0 |
| ∑otherFA | 320.7 | 53.8 | 257.0 | 21.7 | 261.1 | 2.9 | 24.1 | 19.7 | 1.1 | 0.1 | 0.8 | 0.4 | 0.7 | 0.0 | 16.5 | 0.6 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1n-5, 15:1n-6, 16:1n-9, 16:1n-7, Br17:1, 17:1n-8+a17:0, 17:1, 18:1n-9, 18:1n-7, 18:1n-5, 18:1, 19:1, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 22:1n-7, 24:1n-11, 24:1n-9, 24:1n-7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4n-3, 18:3n-6, 18:2n-6, 18:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 21:5n-3, 22:6n-3, 22:5n-3, 22:5n-6, 22:4n-6, 24:6n-3, 24:5n-3; ∑LCn-3 is the sum of 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3, 21:5n-3, 24:6n-3, 24:5n-3∑n-3 is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:6n-3, 22:5n-3, 24:6n-3, 24:5n-3; ∑n-6 is the sum of 18:2n-6, 18:3n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6; ∑otherFA is the sum of individual FA present at <0.1%: 14:1n-5, 15:1n-6c, 16:3+16:4, 16:2a, 16:2b, 16:1n-7t, 16:1n-5c, 16:1n-13t, Br17:1, 17:1n-8c, 17:1, 18:1n-5c, 18:1, 19:1a, 19:1b, 20:4n-6, 20:1n-11c, 20:1n-7c, 20:1n-5c, 21:5n-3, 22:6n-3, 22:4n-6, 22:5n-3, 22:1n-11c, 22:1n-7c, 24:6n-3, 24:1n-11c, 24:5n-3, 24:1n-7c.

*Statistical analysis*

Individual FAs from both feeding trials were combined and initially transformed into percentage total FA format. These data were then analysed using 'Statistical Analysis System' software (SAS Institute., 2009). Summary statistics were calculated with means, standard deviations, minimum and maximum values examined for errors and outliers. Factorial ANOVA (PROC GLM) analysis (SAS Institute., 2009) was then conducted using sire breed, tissue, nutritional plane and sex fitted as fixed effects in the model, and FA values (14:0, 15:0, 16:1n-9c, 16:1n-7c, 16:0, 17:0, 18:2n-6, 18:3n-3, 18:1n-9, 18:1n-7c, 18:1n-7t, 18:0, ARA, 20:5n-3, 20:3n-6, ETA, 20:2n-6, 20:0, DPA-6, DHA, DPA-3, 22:0, 23:0, 24:0, ∑SFA, ∑MUFA, ∑PUFA, ∑n-3, ∑n-6, ∑other FA) as dependent variables. Bonferroni's probability pairwise comparison test was used to separate mean differences, with level of significance as $P<0.05$.

**Results**

Basal diet FA content and composition was described on Table 9.1

*Tissue comparison*

Tissues differed ($P<0.001$) in all FA, except 20:2n-6 ($P>0.119$; Table 9.2). Subcutaneous adipose had the highest ∑SFA composition, including 14:0, 15:0, 16:0, 17:0 and 18:0. Heart ∑PUFA and ∑n-6 PUFA was higher than other tissues, although kidney ∑PUFA composition was similar to heart. Both kidney and liver ∑n-3 PUFA composition was highest. Muscle ∑MUFA composition was highest, followed by subcutaneous adipose, heart and liver, and kidney sequentially. Kidney EPA and DPA composition was highest, whereas ARA was highest in heart tissue and DHA in liver tissue, compared to other tissues analysed. Several FAs were not observed in all tissues.

**Table 9.2** Mean percentage composition of total fatty acids (% total FA), standard error (SEM), number of tissue samples (*n*), and level of significance (*P* value) of subcutaneous adipose (adipose), heart, kidney, liver and muscle tissue from Australian dual-purpose lambs[1,2]

| | Tissue | | | | | | | | | | *P* values |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adipose (*n* 40) | | Heart (*n* 40) | | Kidney (*n* 40) | | Liver (*n* 40) | | Muscle (*n* 40) | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | |
| **% total FA** | | | | | | | | | | | |
| 14:0 | 2.7 | $0.2^{A}$ | 0.8 | $0.1^{C}$ | 0.2 | $0.0^{D}$ | 0.4 | $0.1^{D}$ | 1.7 | $0.1^{B}$ | 0.001 |
| 15:0 | 0.6 | $0.0^{A}$ | 0.4 | $0.0^{C}$ | 0.3 | $0.0^{C}$ | 0.5 | $0.0^{B}$ | 0.4 | $0.0^{B}$ | 0.001 |
| 16:1n-9c | 0.3 | $0.0^{B}$ | 0.2 | $0.0^{C}$ | 0.2 | $0.0^{D}$ | 0.4 | $0.0^{A}$ | 0.3 | $0.0^{C}$ | 0.001 |
| 16:1n-7c | 1.1 | $0.1^{A}$ | 0.4 | $0.0^{C}$ | 0.3 | $0.0^{C}$ | 0.6 | $0.0^{B}$ | 1.2 | $0.1^{A}$ | 0.001 |
| 16:0 | 23.9 | $0.4^{A}$ | 14.7 | $0.3^{D}$ | 18.9 | $0.6^{C}$ | 19.8 | $0.6^{C}$ | 22.4 | $0.3^{B}$ | 0.001 |
| 17:0 | 1.9 | $0.1^{A}$ | 1.3 | $0.0^{B}$ | 1.4 | $0.1^{B}$ | 1.5 | $0.1^{B}$ | 1.4 | $0.0^{B}$ | 0.001 |
| 18:2n-6 | 1.6 | $0.1^{E}$ | 16.3 | $0.7^{A}$ | 9.4 | $0.3^{B}$ | 6.4 | $0.3^{C}$ | 4.1 | $0.2^{D}$ | 0.001 |
| 18:3n-3 | 1.4 | $0.1^{C}$ | 3.2 | $0.2^{A}$ | 2.7 | $0.7^{AB}$ | 3.0 | $0.2^{A}$ | 2.0 | $0.1^{BC}$ | 0.001 |
| 18:1n-9 | 32.8 | $0.9^{B}$ | 19.1 | $0.4^{D}$ | 15.6 | $0.6^{E}$ | 22.2 | $0.6^{C}$ | 35.9 | $0.6^{A}$ | 0.001 |
| 18:1n-7c | 1.3 | $0.0^{C}$ | 2.0 | $0.0^{A}$ | 1.5 | $0.1^{B}$ | 1.3 | $0.0^{C}$ | 1.5 | $0.0^{B}$ | 0.001 |
| 18:1n-7t | 3.6 | $0.2^{A}$ | 2.1 | $0.1^{C}$ | 1.3 | $0.1^{D}$ | 2.2 | $0.2^{C}$ | 2.9 | $0.1^{B}$ | 0.001 |
| 18:0 | 24.0 | $1.1^{A}$ | 20.9 | $0.7^{BC}$ | 20.5 | $0.7^{BC}$ | 22.8 | $0.9^{AB}$ | 19.9 | $0.4^{C}$ | 0.001 |
| 20:4n-6 ARA | 0.0 | $0.0^{C}$ | 4.8 | $0.3^{B}$ | 8.9 | $0.6^{A}$ | 4.0 | $0.4^{B}$ | 0.7 | $0.1^{C}$ | 0.001 |
| 20:5n-3 EPA | 0.0 | $0.0^{C}$ | 2.5 | $0.3^{B}$ | 4.9 | $0.4^{A}$ | 2.9 | $0.3^{B}$ | 0.4 | $0.1^{C}$ | 0.001 |
| 20:3n-6 | 0.0 | $0.0^{D}$ | 0.6 | $0.0^{AB}$ | 0.6 | $0.0^{A}$ | 0.5 | $0.0^{B}$ | 0.1 | $0.0^{C}$ | 0.001 |
| 20:4n-3 | 0.0 | $0.0^{C}$ | 0.1 | $0.0^{A}$ | 0.0 | $0.0^{B}$ | 0.0 | $0.0^{BC}$ | 0.0 | $0.0^{BC}$ | 0.001 |
| 20:2n-6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.119 |
| 20:0 | 0.1 | $0.0^{B}$ | 0.2 | $0.0^{A}$ | 0.2 | $0.0^{A}$ | 0.1 | $0.0^{B}$ | 0.1 | $0.0^{B}$ | 0.001 |
| 22:5n-6 DPA-6 | 0.1 | $0.0^{B}$ | 0.0 | $0.0^{B}$ | 0.3 | $0.1^{A}$ | 0.2 | $0.1^{AB}$ | 0.1 | $0.0^{B}$ | 0.028 |
| 22:6n-3 DHA | 0.0 | $0.0^{D}$ | 1.0 | $0.1^{C}$ | 2.7 | $0.2^{B}$ | 3.3 | $0.3^{A}$ | 0.1 | $0.0^{D}$ | 0.001 |
| 22:5n-3 DPA-3 | 0.0 | $0.0^{C}$ | 1.5 | $0.1^{B}$ | 2.8 | $0.2^{A}$ | 3.2 | $0.2^{A}$ | 0.3 | $0.1^{C}$ | 0.001 |
| 22:0 | 0.0 | $0.0^{C}$ | 0.2 | $0.0^{B}$ | 1.1 | $0.1^{A}$ | 0.2 | $0.1^{B}$ | 0.0 | $0.0^{C}$ | 0.001 |
| 23:0 | 0.0 | $0.0^{B}$ | 0.2 | $0.0^{A}$ | 0.3 | $0.0^{A}$ | 0.2 | $0.0^{A}$ | 0.0 | $0.0^{B}$ | 0.001 |
| 24:0 | 0.0 | $0.0^{C}$ | 0.1 | $0.0^{BC}$ | 0.9 | $0.1^{A}$ | 0.2 | $0.1^{B}$ | 0.0 | $0.0^{C}$ | 0.001 |
| ∑SFA | 54.0 | $1.0^{A}$ | 39.8 | $0.9^{C}$ | 44.5 | $0.7^{B}$ | 46.3 | $0.9^{B}$ | 46.7 | $0.6^{B}$ | 0.001 |
| ∑MUFA | 42.4 | $1.0^{B}$ | 29.5 | $0.5^{C}$ | 22.5 | $0.6^{D}$ | 29.5 | $0.6^{C}$ | 45.3 | $0.6^{A}$ | 0.001 |
| ∑PUFA | 3.6 | $0.1^{D}$ | 30.7 | $1.1^{A}$ | 33.0 | $1.0^{A}$ | 24.2 | $1.2^{B}$ | 8.1 | $0.4^{C}$ | 0.001 |
| ∑n-3 | 1.5 | $0.1^{C}$ | 8.4 | $0.5^{B}$ | 13.3 | $0.8^{A}$ | 12.6 | $0.8^{A}$ | 2.9 | $0.1^{C}$ | 0.001 |
| ∑n-6 | 1.8 | $0.1^{E}$ | 21.9 | $0.9^{A}$ | 19.4 | $0.8^{B}$ | 11.3 | $0.6^{C}$ | 5.0 | $0.3^{D}$ | 0.001 |
| ∑other FA | 4.3 | $0.2^{C}$ | 7.4 | $0.3^{A}$ | 5.0 | $0.2^{B}$ | 4.2 | $0.1^{C}$ | 4.6 | $0.3^{BC}$ | 0.001 |
| **mg/100g** | | | | | | | | | | | |
| 20:4n-6 ARA | 10.0 | 2.5 | 62.8 | 4.0 | 82.5 | 10.1 | 83.0 | 9.8 | 12.9 | 1.5 | 0.001 |
| 20:5n-3 EPA | 19.0 | 7.0 | 32.8 | 2.8 | 48.0 | 6.1 | 68.6 | 9.8 | 9.7 | 1.4 | 0.001 |
| 22:5n-6 DPA-6 | 24.7 | 8.7 | 0.4 | 0.1 | 1.1 | 0.4 | 3.5 | 1.9 | 1.4 | 0.9 | 0.001 |
| 22:6n-3 DHA | 14.6 | 10.8 | 12.7 | 1.0 | 25.5 | 3.1 | 69.3 | 7.0 | 2.0 | 0.4 | 0.001 |
| 22:5n-3 DPA-3 | 16.6 | 4.8 | 21.0 | 1.8 | 27.7 | 3.6 | 75.6 | 8.9 | 6.1 | 1.3 | 0.001 |

[1] Means with different superscripts [A, B, C, D, E] within rows significantly differ ($P<0.05$).

[2] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1n-5, 15:1n-6, 16:1n-9, 16:1n-7, Br17:1, 17:1n-8+a17:0, 17:1, 18:1n-9, 18:1n-7, 18:1n-5, 18:1, 19:1, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 22:1n-7, 24:1n-11, 24:1n-9, 24:1n-7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4n-3, 18:3n-6, 18:2n-6, 18:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 21:5n-3, 22:6n-3, 22:5n-3, 22:5n-6, 22:4n-6, 24:6n-3, 24:5n-3;∑n-3 PUFA is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:6n-3, 22:5n-3, 24:6n-3, 24:5n-3; ∑n-6 PUFA is the sum of 18:2n-6, 18:3n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6; ∑otherFA is the sum of 14:1n-5, 15:1n-6, 16:3+16:4, 16:2, 16:1n-5, 16:1n-13, Br17:1, 17:1n-8+a17:0, 18:4n-3, 18:1, 20:2n-6, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 21:5n-3, 21:0, 22:4n-6, 22:1n-9, 22:1n-11, 22:1n-7, 24:6n-3, 24:5n-3, 24:1n-11, 24:1n-9, 24:1n-7.

*Effect of sex*

Ewe heart 22:5n-6 composition was greater than wethers (Table 9.4). Muscle 18:1n-9 composition was higher in wethers than ewes ($P$<0.016; Table 9.7). All other sex effects on FA profile had no significance ($P$>0.05; Table 9.2; Table 9.4; Table 9.6).

*Effect of sire breed*

Black Suffolk- and Merino-sired lambs had the highest subcutaneous adipose 16:1n-9c composition, followed by White Suffolk- and Dorset-sired lambs respectively. For Black Suffolk-sired lambs, subcutaneous adipose 17:0 and ∑other FA composition was highest (Table 9.3). In Merino-sired lambs, heart 16:0 composition was highest, followed by Dorset-, White Suffolk- and Black Suffolk-sired lambs sequentially ($P$>0.006; Table 9.3). Kidney 16:1n-7c composition of Merino-sired lambs was higher compared to White Suffolk- and Black Suffolk-sired lambs ($P$<0.005; Table 9.5). Kidney 16:0 and 18:3n-3 composition of Merino sired lambs was highest, with other sire breed's composition not differing significantly. Kidney DHA composition was higher in Dorset- than Black Suffolk- and Merino-sired lambs ($P$<0.026). Merino-sired lambs liver 16:0 composition of 23.6 ± 2.3 % was highest, followed by Dorset- and White Suffolk-, and Black Suffolk-sired lambs respectively ($P$<0.043; Table 9.6). Black Suffolk- and Merino-sired lambs muscle 17:0 composition was higher than Dorset- and White Suffolk-sired lambs ($P$<0.001; Table 9.7). Muscle 18:3n-3 composition was lowest in Dorset-sired lambs comparative to Black Suffolk- and Merino-sired lamb counterparts ($P$<0.015). Muscle 18:1n-7t composition of 1.7 ± 0.1 % was highest ($P$<0.001) in Black Suffolk-sired lambs, with levels for other sire breeds not significantly different. All other sire breed effects on FA profiles of analysed tissues were shown to have no significance ($P$>0.05).

**Table 9.3** Mean percentage of total fatty acid (% total FA), standard error (SEM), number of tissue samples (*n*), and level of significance (*P* value) of subcutaneous adipose tissue in Australian dual-purpose lambs with different sex (ewe, wether) and sire breeds[1,2]

| | Sex (S) | | | | Breed (B) | | | | | | | | *P* values | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe (*n* 17) | | Wether (*n* 23) | | Black Suffolk (*n* 6) | | Dorset (*n* 14) | | Merino (*n* 6) | | White Suffolk (*n* 14) | | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | S | B |
| **% total FA** | | | | | | | | | | | | | | |
| 14:0 | 2.6 | 0.3 | 2.7 | 0.2 | 2.5 | 0.6 | 2.4 | 0.3 | 3.3 | 4.2 | 2.8 | 0.3 | 0.575 | 0.449 |
| 15:0 | 0.7 | 0.1 | 0.6 | 0.1 | 0.7 | 0.1 | 0.6 | 0.1 | 0.7 | 0.1 | 0.7 | 0.1 | 0.655 | 0.921 |
| 16:1n-9c | 0.3 | 0.0 | 0.4 | 0.0 | 0.4 | $0.0^{A}$ | 0.3 | $0.0^{B}$ | 0.4 | $0.0^{A}$ | 0.4 | $0.0^{AB}$ | 0.262 | 0.030 |
| 16:1n-7c | 1.2 | 0.1 | 1.1 | 0.1 | 1.3 | 0.1 | 1.1 | 0.2 | 0.7 | 0.2 | 1.3 | 0.2 | 0.849 | 0.431 |
| 16:0 | 23.8 | 0.7 | 24.0 | 0.3 | 24.1 | 0.5 | 23.6 | 0.8 | 24.4 | 1.0 | 23.9 | 0.5 | 0.966 | 0.736 |
| 17:0 | 1.9 | 0.1 | 1.9 | 0.1 | 2.5 | $0.2^{A}$ | 1.9 | $0.1^{B}$ | 2.0 | $0.1^{B}$ | 1.8 | $0.1^{B}$ | 0.453 | 0.002 |
| 18:2n-6 | 1.5 | 0.1 | 1.7 | 0.1 | 1.6 | 0.1 | 1.5 | 0.1 | 2.1 | 0.2 | 1.5 | 0.1 | 0.971 | 0.066 |
| 18:3n-3 | 1.3 | 0.1 | 1.5 | 0.1 | 1.4 | 0.1 | 1.3 | 0.1 | 1.6 | 0.1 | 1.4 | 0.1 | 0.313 | 0.464 |
| 18:1n-9 | 32.7 | 1.6 | 32.9 | 1.1 | 32.9 | 1.3 | 32.7 | 2.0 | 29.0 | 1.8 | 34.5 | 1.1 | 0.648 | 0.236 |
| 18:1n-7c | 1.2 | 0.1 | 1.3 | 0.0 | 1.6 | 0.1 | 1.1 | 0.1 | 1.2 | 0.0 | 1.3 | 0.0 | 0.343 | 0.095 |
| 18:1n-7t | 3.5 | 0.2 | 3.7 | 0.2 | 4.3 | 0.3 | 3.2 | 0.2 | 3.6 | 0.5 | 3.7 | 0.2 | 0.613 | 0.341 |
| 18:0 | 24.6 | 2.4 | 23.5 | 1.0 | 21.0 | 2.0 | 26.0 | 2.8 | 26.8 | 1.6 | 22.1 | 0.9 | 0.607 | 0.302 |
| 20:4n-6 ARA | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.127 | 0.288 |
| 20:5n-3 EPA | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.566 | 0.309 |
| 20:2n-6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.767 | 0.815 |
| 20:0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.708 | 0.832 |
| 22:5n-6 DPA-6 | 0.1 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.3 | 0.2 | 0.0 | 0.0 | 0.669 | 0.546 |
| 22:6n-3 DHA | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.702 | 0.386 |
| 22:5n-3 DPA-3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.443 | 0.421 |
| ∑SFA | 54.5 | 1.9 | 53.6 | 1.1 | 51.4 | 1.5 | 55.5 | 2.3 | 58.0 | 1.8 | 51.9 | 1.1 | 0.431 | 0.141 |
| ∑MUFA | 42.1 | 1.9 | 42.6 | 1.1 | 45.2 | 1.4 | 41.3 | 2.3 | 37.7 | 1.9 | 44.4 | 1.1 | 0.485 | 0.137 |
| ∑PUFA | 3.3 | 0.2 | 3.7 | 0.2 | 3.4 | 0.3 | 3.2 | 0.2 | 4.3 | 0.3 | 3.7 | 0.2 | 0.553 | 0.310 |
| ∑n-3 | 1.4 | 0.1 | 1.6 | 0.1 | 1.4 | 0.1 | 1.4 | 0.1 | 1.7 | 0.1 | 1.7 | 0.2 | 0.270 | 0.187 |
| ∑n-6 | 1.7 | 0.1 | 1.8 | 0.1 | 1.7 | 0.1 | 1.6 | 0.1 | 2.4 | 0.2 | 1.7 | 0.1 | 0.925 | 0.088 |
| ∑other FA | 4.3 | 0.3 | 4.3 | 0.2 | 5.6 | $0.4^{A}$ | 4.0 | $0.3^{B}$ | 3.8 | $0.2^{B}$ | 4.2 | $0.2^{B}$ | 0.430 | 0.036 |
| **mg/100g** | | | | | | | | | | | | | | |
| 20:4n-6 ARA | 9.1 | 3.2 | 10.8 | 3.8 | 8.5 | 8.5 | 9.3 | 3.7 | 6.4 | 6.4 | 13.0 | 4.6 | 0.558 | 0.793 |
| 20:5n-3 EPA | 12.9 | 6.9 | 23.5 | 11.0 | 0.0 | 0.0 | 20.8 | 12.3 | 2.3 | 2.3 | 32.6 | 15.1 | 0.234 | 0.251 |
| 22:5n-6 DPA-6 | 23.3 | 11.4 | 25.8 | 12.8 | 0.0 | 0.0 | 22.7 | 10.9 | 58.6 | 46.4 | 22.8 | 10.8 | 0.663 | 0.311 |
| 22:6n-3 DHA | 6.1 | 4.4 | 20.9 | 18.5 | 0.0 | 0.0 | 4.5 | 2.6 | 0.0 | 0.0 | 37.2 | 30.5 | 0.356 | 0.438 |
| 22:5n-3 DPA-3 | 13.7 | 7.1 | 18.8 | 6.6 | 0.0 | 0.0 | 22.1 | 9.1 | 8.4 | 3.2 | 21.8 | 9.8 | 0.407 | 0.332 |

[1] Means with different superscripts $^{A, B, C, D, E}$ within rows significantly differ ($P<0.05$).
[2] as per Table 2.

**Table 9.4** Mean percentage of total fatty acid (% total FA), standard error (SEM), number of tissue samples (*n*), and level of significance (*P* value) of heart tissue in Australian dual-purpose lambs with different sex (ewe, wether) and sire breeds [1,2]

| | Sex (S) | | | | Breed (B) | | | | | | | | *P* values | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe (*n* 17) | | Wether (*n* 23) | | Black Suffolk (*n* 6) | | Dorset (*n* 14) | | Merino (*n* 6) | | White Suffolk (*n* 14) | | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | S | B |
| **% total FA** | | | | | | | | | | | | | | |
| 14:0 | 0.8 | 0.1 | 0.7 | 0.1 | 0.8 | 0.2 | 0.8 | 0.2 | 1.1 | 0.3 | 0.5 | 0.1 | 0.475 | 0.143 |
| 15:0 | 0.3 | 0.0 | 0.4 | 0.0 | 0.4 | 0.0 | 0.4 | 0.1 | 0.3 | 0.1 | 0.3 | 0.0 | 0.204 | 0.653 |
| 16:1n-9c | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 | 0.323 | 0.113 |
| 16:1n-7c | 0.3 | 0.1 | 0.4 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 | 0.4 | 0.1 | 0.3 | 0.1 | 0.986 | 0.983 |
| 16:0 | 14.6 | 0.3 | 14.8 | 0.4 | 13.4 | $0.4^{C}$ | 15.1 | $0.4^{B}$ | 16.5 | $0.8^{A}$ | 14.2 | $0.3^{BC}$ | 0.533 | 0.006 |
| 17:0 | 1.3 | 0.0 | 1.4 | 0.0 | 1.3 | 0.1 | 1.4 | 0.0 | 1.4 | 0.1 | 1.3 | 0.0 | 0.814 | 0.103 |
| 18:2n-6 | 15.4 | 1.0 | 16.9 | 1.0 | 16.1 | 1.6 | 14.2 | 1.1 | 17.5 | 2.1 | 17.9 | 1.1 | 0.346 | 0.089 |
| 18:3n-3 | 2.8 | 0.2 | 3.5 | 0.2 | 3.7 | 0.3 | 2.7 | 0.2 | 3.3 | 0.4 | 3.4 | 0.4 | 0.066 | 0.242 |
| 18:1n-9 | 19.7 | 0.7 | 18.7 | 0.5 | 19.0 | 1.7 | 19.7 | 0.8 | 20.2 | 0.9 | 18.2 | 0.6 | 0.156 | 0.251 |
| 18:1n-7c | 2.0 | 0.1 | 1.9 | 0.1 | 2.0 | 0.1 | 1.9 | 0.1 | 2.0 | 0.1 | 2.0 | 0.1 | 0.358 | 0.467 |
| 18:1n-7t | 2.2 | 0.2 | 2.1 | 0.1 | 2.6 | 0.2 | 2.1 | 0.2 | 2.2 | 0.2 | 1.9 | 0.2 | 0.694 | 0.632 |
| 18:0 | 21.4 | 1.0 | 20.5 | 0.9 | 21.1 | 0.7 | 22.2 | 1.3 | 20.1 | 1.7 | 19.7 | 1.0 | 0.903 | 0.338 |
| 20:4n-6 ARA | 4.7 | 0.5 | 4.8 | 0.4 | 4.6 | 0.3 | 4.7 | 0.7 | 4.1 | 0.6 | 5.3 | 0.5 | 0.874 | 0.635 |
| 20:5n-3 EPA | 2.4 | 0.4 | 2.6 | 0.3 | 3.2 | 0.3 | 2.3 | 0.5 | 1.9 | 0.3 | 2.7 | 0.5 | 0.708 | 0.848 |
| 20:3n-6 | 0.5 | 0.0 | 0.6 | 0.0 | 0.6 | 0.0 | 0.5 | 0.0 | 0.5 | 0.1 | 0.6 | 0.0 | 0.408 | 0.261 |
| 20:4n-3 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.288 | 0.797 |
| 20:2n-6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.579 | 0.385 |
| 20:0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.936 | 0.378 |
| 22:5n-6 DPA-6 | 0.0 | $0.0^{A}$ | 0.0 | $0.0^{B}$ | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.008 | 0.565 |
| 22:6n-3 DHA | 1.0 | 0.2 | 1.0 | 0.1 | 0.8 | 0.1 | 1.0 | 0.2 | 0.7 | 0.1 | 1.1 | 0.2 | 0.741 | 0.533 |
| 22:5n-3 DPA-3 | 1.5 | 0.2 | 1.6 | 0.1 | 1.5 | 0.4 | 1.5 | 0.2 | 1.0 | 0.1 | 1.8 | 0.1 | 0.400 | 0.233 |
| 22:0 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.0 | 0.3 | 0.1 | 0.1 | 0.0 | 0.3 | 0.1 | 0.825 | 0.514 |
| 23:0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.1 | 0.2 | 0.0 | 0.1 | 0.1 | 0.2 | 0.1 | 0.737 | 0.788 |
| 24:0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 | 0.832 | 0.351 |
| ∑SFA | 40.2 | 1.3 | 39.4 | 1.2 | 38.5 | 1.1 | 41.7 | 1.7 | 40.9 | 2.6 | 37.9 | 1.3 | 0.753 | 0.171 |
| ∑MUFA | 30.3 | 0.9 | 28.9 | 0.6 | 30.5 | 1.5 | 30.1 | 1.0 | 26.5 | 1.1 | 28.4 | 0.8 | 0.149 | 0.457 |
| ∑PUFA | 29.5 | 1.8 | 31.7 | 1.5 | 31.0 | 2.3 | 28.1 | 2.1 | 29.6 | 3.3 | 33.7 | 1.7 | 0.340 | 0.162 |
| ∑n-3 | 8.0 | 0.7 | 8.7 | 0.6 | 9.2 | 1.0 | 7.8 | 0.8 | 7.1 | 0.7 | 9.1 | 0.9 | 0.297 | 0.360 |
| ∑n-6 | 21.0 | 1.4 | 22.6 | 1.2 | 21.5 | 1.7 | 19.7 | 1.6 | 22.2 | 2.7 | 24.0 | 1.4 | 0.420 | 0.125 |
| ∑other FA | 7.8 | 0.7 | 7.2 | 0.3 | 7.7 | 0.5 | 7.8 | 0.9 | 6.0 | 0.5 | 7.6 | 0.4 | 0.534 | 0.778 |
| **mg/100g** | | | | | | | | | | | | | | |
| 20:4n-6 ARA | 67.4 | 7.1 | 59.4 | 4.5 | 69.2 | 9.0 | 66.3 | 6.6 | 50.7 | 7.5 | 61.7 | 7.9 | 0.570 | 0.719 |
| 20:5n-3 EPA | 34.1 | 4.2 | 31.9 | 3.9 | 46.7 | 6.7 | 33.3 | 5.2 | 24.8 | 5.2 | 29.9 | 4.5 | 0.956 | 0.175 |
| 22:5n-6 DPA-6 | 0.7 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.6 | 0.2 | 0.2 | 0.2 | 0.5 | 0.2 | 0.055 | 0.628 |
| 22:6n-3 DHA | 13.6 | 1.9 | 12.1 | 1.1 | 11.7 | 2.1 | 14.4 | 2.0 | 9.2 | 1.6 | 13.0 | 1.8 | 0.827 | 0.506 |
| 22:5n-3 DPA-3 | 21.9 | 3.2 | 20.3 | 2.2 | 23.5 | 5.8 | 21.2 | 2.6 | 14.8 | 4.8 | 22.3 | 3.3 | 0.939 | 0.605 |

[1] Means with different superscripts $^{A, B, C, D, E}$ within rows significantly differ ($P<0.05$).
[2] as per Table 2.

**Table 9.5** Mean percentage of total fatty acid (% total FA), standard error (SEM), number of tissue samples (*n*), and level of significance (*P* value) of kidney tissue in Australian dual-purpose lambs with different sex (ewe, wether) and sire breeds [1,2]

| | Sex (S) | | | | Breed (B) | | | | | | | | *P* values | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe (*n* 17) | | Wether (*n* 23) | | Black Suffolk (*n* 6) | | Dorset (*n* 14) | | Merino (*n* 6) | | White Suffolk (*n* 14) | | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | S | B |
| **% total FA** | | | | | | | | | | | | | | |
| 14:0 | 0.2 | 0.1 | 0.2 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.4 | 0.2 | 0.2 | 0.1 | 0.347 | 0.089 |
| 15:0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.4 | 0.1 | 0.3 | 0.0 | 0.2 | 0.1 | 0.3 | 0.0 | 0.090 | 0.232 |
| 16:1n-9c | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.3 | 0.1 | 0.2 | 0.0 | 0.340 | 0.256 |
| 16:1n-7c | 0.3 | 0.0 | 0.3 | 0.1 | 0.2 | $0.1^{B}$ | 0.3 | $0.1^{AB}$ | 0.5 | $0.2^{A}$ | 0.3 | $0.0^{B}$ | 0.863 | 0.005 |
| 16:0 | 18.3 | 0.4 | 19.3 | 0.9 | 17.6 | $0.7^{B}$ | 17.6 | $0.5^{B}$ | 23.1 | $3.0^{A}$ | 19.0 | $0.5^{B}$ | 0.903 | 0.005 |
| 17:0 | 1.3 | 0.1 | 1.4 | 0.1 | 1.3 | 0.1 | 1.3 | 0.1 | 1.3 | 0.2 | 1.4 | 0.1 | 0.754 | 0.067 |
| 18:2n-6 | 9.1 | 0.3 | 9.6 | 0.4 | 8.9 | 0.9 | 9.3 | 0.4 | 9.6 | 1.1 | 9.5 | 0.3 | 0.199 | 0.355 |
| 18:3n-3 | 1.8 | 0.1 | 3.3 | 1.2 | 2.3 | $0.3^{B}$ | 1.8 | $0.2^{B}$ | 7.0 | $4.6^{A}$ | 1.8 | $0.2^{B}$ | 0.816 | 0.001 |
| 18:1n-9 | 15.1 | 0.3 | 16.0 | 1.1 | 17.4 | 2.6 | 15.4 | 0.8 | 15.8 | 2.7 | 15.0 | 0.4 | 0.296 | 0.638 |
| 18:1n-7c | 1.4 | 0.0 | 1.5 | 0.1 | 1.1 | 0.2 | 1.5 | 0.1 | 1.7 | 0.3 | 1.5 | 0.1 | 0.994 | 0.122 |
| 18:1n-7t | 1.2 | 0.2 | 1.3 | 0.2 | 2.0 | 0.6 | 1.2 | 0.2 | 1.1 | 0.4 | 1.2 | 0.2 | 0.272 | 0.561 |
| 18:0 | 20.8 | 0.6 | 20.2 | 1.1 | 21.6 | 0.7 | 20.6 | 0.9 | 18.3 | 3.5 | 20.7 | 0.9 | 0.848 | 0.229 |
| 20:4n-6 ARA | 9.9 | 0.7 | 8.2 | 1.0 | 7.5 | 1.8 | 10.1 | 1.1 | 5.6 | 1.7 | 9.7 | 0.9 | 0.247 | 0.117 |
| 20:5n-3 EPA | 5.4 | 0.6 | 4.5 | 0.6 | 6.3 | 1.3 | 5.3 | 0.7 | 2.2 | 0.7 | 5.0 | 0.7 | 0.744 | 0.067 |
| 20:3n-6 | 0.7 | 0.1 | 0.6 | 0.1 | 0.5 | 0.1 | 0.7 | 0.1 | 0.5 | 0.1 | 0.7 | 0.1 | 0.736 | 0.495 |
| 20:4n-3 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.159 | 0.696 |
| 20:2n-6 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.339 | 0.630 |
| 20:0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 | 0.2 | 0.0 | 0.115 | 0.345 |
| 22:5n-6 DPA-6 | 0.3 | 0.1 | 0.3 | 0.1 | 0.0 | 0.0 | 0.3 | 0.1 | 0.5 | 0.3 | 0.4 | 0.2 | 0.849 | 0.955 |
| 22:6n-3 DHA | 2.9 | 0.2 | 2.6 | 0.2 | 2.0 | $0.4^{B}$ | 3.4 | $0.2^{A}$ | 1.9 | $0.4^{B}$ | 2.7 | $0.2^{AB}$ | 0.434 | 0.026 |
| 22:5n-3 DPA-3 | 3.1 | 0.2 | 2.6 | 0.2 | 3.0 | 0.6 | 3.0 | 0.2 | 2.0 | 0.5 | 2.9 | 0.3 | 0.157 | 0.453 |
| 22:0 | 1.1 | 0.1 | 1.1 | 0.2 | 1.3 | 0.3 | 1.2 | 0.2 | 1.0 | 0.4 | 1.0 | 0.1 | 0.932 | 0.898 |
| 23:0 | 0.2 | 0.0 | 0.3 | 0.1 | 0.1 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 0.0 | 0.888 | 0.865 |
| 24:0 | 0.8 | 0.1 | 1.0 | 0.2 | 0.8 | 0.2 | 1.0 | 0.3 | 0.9 | 0.4 | 0.8 | 0.1 | 0.864 | 0.858 |
| ∑SFA | 44.1 | 0.9 | 44.8 | 1.1 | 44.3 | 0.5 | 43.3 | 1.2 | 47.1 | 2.1 | 44.7 | 1.5 | 0.978 | 0.638 |
| ∑MUFA | 22.1 | 0.5 | 22.9 | 1.0 | 24.7 | 2.7 | 22.1 | 0.9 | 22.8 | 2.2 | 21.9 | 0.5 | 0.303 | 0.765 |
| ∑PUFA | 33.8 | 1.2 | 32.3 | 1.4 | 31.1 | 3.0 | 34.6 | 1.6 | 30.2 | 2.2 | 33.3 | 1.6 | 0.497 | 0.695 |
| ∑n-3 | 13.4 | 0.8 | 13.2 | 1.2 | 13.9 | 2.1 | 13.7 | 0.8 | 13.3 | 3.5 | 12.5 | 1.1 | 0.612 | 0.198 |
| ∑n-6 | 20.2 | 0.9 | 18.8 | 1.2 | 17.1 | 1.3 | 20.5 | 1.4 | 16.3 | 2.9 | 20.5 | 1.0 | 0.486 | 0.051 |
| ∑other FA | 5.1 | 0.1 | 5.0 | 0.3 | 5.1 | 0.4 | 4.8 | 0.3 | 5.4 | 0.9 | 5.1 | 0.3 | 0.513 | 0.478 |
| **mg/100g** | | | | | | | | | | | | | | |
| 20:4n-6 ARA | 107.9 | 17.2 | 63.7 | 10.8 | 90.0 | 37.9 | 77.6 | 14.4 | 57.3 | 25.3 | 94.9 | 16.8 | 0.058 | 0.885 |
| 20:5n-3 EPA | 64.2 | 11.2 | 36.0 | 5.6 | 63.8 | 18.9 | 44.7 | 10.3 | 27.9 | 11.9 | 53.0 | 10.2 | 0.053 | 0.673 |
| 22:5n-6 DPA-6 | 1.4 | 0.7 | 0.9 | 0.3 | 0.1 | 0.1 | 1.6 | 0.9 | 1.2 | 0.8 | 1.1 | 0.4 | 0.500 | 0.625 |
| 22:6n-3 DHA | 30.5 | 4.7 | 21.7 | 3.9 | 22.4 | 8.6 | 28.2 | 6.1 | 20.7 | 8.1 | 26.0 | 4.3 | 0.219 | 0.943 |
| 22:5n-3 DPA-3 | 34.2 | 5.8 | 22.9 | 4.3 | 32.8 | 12.6 | 26.4 | 6.3 | 22.5 | 9.4 | 29.1 | 5.2 | 0.166 | 0.952 |

[1] Means with different superscripts $^{A, B, C, D, E}$ within rows significantly differ ($P<0.05$).

[2] as per Table 2.

**Table 9.6** Mean percentage of total fatty acid (% total FA), standard error (SEM), number of tissue samples (*n*), and level of significance (*P* value) of liver tissue in Australian dual-purpose lambs with different sex (ewe, wether) and sire breeds [1,2]

| | Sex (S) | | | | Breed (B) | | | | | | | | *P* values | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe (*n* 17) | | Wether (*n* 23) | | Black Suffolk (*n* 6) | | Dorset (*n* 14) | | Merino (*n* 6) | | White Suffolk (*n* 14) | | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | S | B |
| **% total FA** | | | | | | | | | | | | | | |
| 14:0 | 0.3 | 0.1 | 0.5 | 0.1 | 0.5 | 0.0 | 0.4 | 0.1 | 0.7 | 0.4 | 0.3 | 0.1 | 0.619 | 0.577 |
| 15:0 | 0.4 | 0.1 | 0.5 | 0.0 | 0.4 | 0.0 | 0.4 | 0.1 | 0.6 | 0.1 | 0.5 | 0.1 | 0.517 | 0.511 |
| 16:1n-9c | 0.4 | 0.0 | 0.5 | 0.0 | 0.4 | 0.0 | 0.4 | 0.1 | 0.6 | 0.1 | 0.4 | 0.0 | 0.768 | 0.077 |
| 16:1n-7c | 0.6 | 0.1 | 0.6 | 0.1 | 0.7 | 0.1 | 0.6 | 0.0 | 0.6 | 0.2 | 0.6 | 0.1 | 0.635 | 0.871 |
| 16:0 | 19.5 | 0.8 | 20.0 | 0.9 | 16.9 | 0.6$^{C}$ | 19.2 | 0.7$^{B}$ | 23.6 | 2.3$^{A}$ | 19.9 | 0.9$^{B}$ | 0.426 | 0.043 |
| 17:0 | 1.5 | 0.1 | 1.5 | 0.1 | 1.3 | 0.0 | 1.5 | 0.1 | 1.5 | 0.2 | 1.5 | 0.1 | 0.873 | 0.645 |
| 18:2n-6 | 6.2 | 0.3 | 6.5 | 0.4 | 5.6 | 0.2 | 6.2 | 0.5 | 7.7 | 0.4 | 6.2 | 0.4 | 0.826 | 0.328 |
| 18:3n-3 | 2.9 | 0.2 | 3.1 | 0.2 | 3.2 | 0.2 | 2.8 | 0.3 | 3.6 | 0.6 | 2.9 | 0.3 | 0.989 | 0.795 |
| 18:1n-9 | 22.9 | 0.9 | 21.7 | 0.7 | 22.3 | 1.1 | 22.0 | 0.8 | 23.7 | 1.8 | 21.8 | 1.1 | 0.090 | 0.170 |
| 18:1n-7c | 1.2 | 0.1 | 1.3 | 0.0 | 1.4 | 0.0 | 1.1 | 0.1 | 1.2 | 0.1 | 1.3 | 0.1 | 0.672 | 0.079 |
| 18:1n-7t | 2.2 | 0.3 | 2.1 | 0.2 | 2.5 | 0.7 | 2.1 | 0.2 | 2.3 | 0.3 | 2.1 | 0.3 | 0.734 | 0.909 |
| 18:0 | 23.3 | 1.7 | 22.3 | 1.1 | 24.5 | 0.9 | 23.6 | 2.1 | 18.7 | 1.8 | 22.9 | 1.2 | 0.497 | 0.331 |
| 20:4n-6 ARA | 4.2 | 0.6 | 4.0 | 0.5 | 3.6 | 0.2 | 4.5 | 0.6 | 3.4 | 1.3 | 4.1 | 0.7 | 0.869 | 0.781 |
| 20:5n-3 EPA | 2.8 | 0.4 | 2.9 | 0.4 | 3.9 | 0.1 | 2.7 | 0.5 | 2.4 | 1.2 | 2.8 | 0.5 | 0.817 | 0.889 |
| 20:3n-6 | 0.5 | 0.1 | 0.5 | 0.0 | 0.5 | 0.1 | 0.5 | 0.1 | 0.4 | 0.1 | 0.5 | 0.1 | 0.433 | 0.627 |
| 20:0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.228 | 0.929 |
| 22:5n-6 DPA-6 | 0.1 | 0.0 | 0.3 | 0.2 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.1 | 0.4 | 0.3 | 0.293 | 0.669 |
| 22:6n-3 DHA | 3.3 | 0.4 | 3.3 | 0.4 | 3.2 | 0.3 | 3.8 | 0.6 | 1.6 | 0.4 | 3.6 | 0.4 | 0.594 | 0.126 |
| 22:5n-3 DPA-3 | 3.1 | 0.4 | 3.3 | 0.3 | 4.2 | 0.5 | 3.1 | 0.4 | 2.1 | 0.6 | 3.3 | 0.4 | 0.449 | 0.363 |
| 22:0 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 | 0.3 | 0.3 | 0.2 | 0.1 | 0.896 | 0.204 |
| 23:0 | 0.1 | 0.0 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.0 | 0.296 | 0.589 |
| 24:0 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.0 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.620 | 0.545 |
| ∑SFA | 46.2 | 1.5 | 46.3 | 1.2 | 44.7 | 0.8 | 46.6 | 2.0 | 46.6 | 2.9 | 46.6 | 1.4 | 0.634 | 0.956 |
| ∑MUFA | 30.1 | 0.9 | 29.2 | 0.8 | 30.4 | 1.2 | 29.0 | 0.9 | 31.4 | 2.0 | 28.9 | 1.1 | 0.145 | 0.114 |
| ∑PUFA | 23.7 | 1.9 | 24.5 | 1.6 | 24.9 | 1.1 | 24.4 | 2.4 | 22.0 | 3.6 | 24.5 | 2.0 | 0.760 | 0.897 |
| ∑n-3 | 12.4 | 1.2 | 12.8 | 1.1 | 14.9 | 0.8 | 12.6 | 1.5 | 9.8 | 2.1 | 12.9 | 1.5 | 0.637 | 0.683 |
| ∑n-6 | 11.1 | 0.9 | 11.5 | 0.8 | 9.9 | 0.5 | 11.6 | 1.2 | 11.8 | 1.5 | 11.4 | 1.0 | 0.956 | 0.823 |
| ∑other FA | 4.0 | 0.2 | 4.3 | 0.1 | 4.6 | 0.1 | 4.1 | 0.2 | 4.4 | 0.3 | 4.0 | 0.2 | 0.545 | 0.829 |
| **mg/100g** | | | | | | | | | | | | | | |
| 20:4n-6 ARA | 74.2 | 10.3 | 89.4 | 15.4 | 110.7 | 5.9 | 91.1 | 22.5 | 76.5 | 29.9 | 65.7 | 10.6 | 0.364 | 0.433 |
| 20:5n-3 EPA | 60.8 | 12.7 | 74.3 | 14.3 | 120.1 | 7.0 | 64.8 | 19.4 | 57.6 | 28.3 | 55.1 | 13.9 | 0.352 | 0.142 |
| 22:5n-6 DPA-6 | 1.7 | 1.0 | 4.9 | 3.2 | 0.0 | 0.0 | 1.8 | 0.7 | 2.3 | 1.5 | 7.3 | 5.3 | 0.328 | 0.487 |
| 22:6n-3 DHA | 68.6 | 11.9 | 69.9 | 8.7 | 98.8 | 7.3 | 71.9 | 13.4 | 36.3 | 9.0 | 68.3 | 12.4 | 0.372 | 0.079 |
| 22:5n-3 DPA-3 | 69.9 | 13.7 | 79.9 | 11.8 | 130.0 | 15.5$^{A}$ | 68.8 | 15.5$^{B}$ | 53.0 | 19.0$^{B}$ | 68.8 | 14.6$^{B}$ | 0.289 | 0.048 |

[1] Means with different superscripts $^{A, B, C, D, E}$ within rows significantly differ ($P<0.05$).

[2] FA not found (%total FA = 0) were 20:4ω3, 20:2ω6.

**Table 9.7** Mean percentage of total fatty acid (% total FA), standard error (SEM), number of tissue samples (*n*), and level of significance (*P* value) of *Longissimus dorsi* muscle tissue in Australian dual-purpose lambs with different sex (ewe, wether) and sire breeds [1,2]

| | Sex (S) | | | | Breed (B) | | | | | | | | *P* values | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe (*n* 17) | | Wether (*n* 23) | | Black Suffolk (*n* 6) | | Dorset (*n* 14) | | Merino (*n* 6) | | White Suffolk (*n* 14) | | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | S | B |
| **% total FA** | | | | | | | | | | | | | | |
| 14:0 | 1.7 | 0.2 | 1.7 | 0.2 | 2.2 | 0.2 | 1.6 | 0.2 | 1.6 | 0.4 | 1.7 | 0.2 | 0.823 | 0.962 |
| 15:0 | 0.4 | 0.0 | 0.4 | 0.0 | 0.5 | 0.0 | 0.4 | 0.0 | 0.5 | 0.1 | 0.4 | 0.0 | 0.898 | 0.627 |
| 16:1n-9c | 0.3 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.606 | 0.244 |
| 16:1n-7c | 1.2 | 0.1 | 1.1 | 0.1 | 1.3 | 0.1 | 1.2 | 0.0 | 1.2 | 0.1 | 1.0 | 0.1 | 0.112 | 0.375 |
| 16:0 | 22.9 | 0.5 | 22.1 | 0.4 | 22.8 | 1.0 | 22.1 | 0.4 | 22.9 | 1.0 | 22.4 | 0.5 | 0.099 | 0.723 |
| 17:0 | 1.4 | 0.1 | 1.4 | 0.0 | 1.6 | $0.1^{A}$ | 1.3 | $0.0^{B}$ | 1.5 | $0.1^{A}$ | 1.3 | $0.0^{B}$ | 0.995 | 0.001 |
| 18:2n-6 | 3.9 | 0.4 | 4.2 | 0.3 | 3.7 | 0.2 | 3.8 | 0.4 | 5.0 | 0.5 | 4.1 | 0.4 | 0.535 | 0.288 |
| 18:3n-3 | 1.9 | 0.1 | 2.0 | 0.1 | 2.2 | $0.1^{A}$ | 1.8 | $0.1^{B}$ | 2.2 | $0.2^{A}$ | 2.0 | $0.1^{AB}$ | 0.459 | 0.015 |
| 18:1n-9 | 35.3 | $1.0^{B}$ | 36.4 | $0.6^{A}$ | 35.3 | 2.4 | 36.7 | 0.7 | 33.6 | 0.9 | 36.5 | 0.9 | 0.016 | 0.061 |
| 18:1n-7c | 1.4 | 0.1 | 1.5 | 0.0 | 1.7 | $0.1^{A}$ | 1.4 | $0.0^{B}$ | 1.4 | $0.1^{B}$ | 1.5 | $0.0^{B}$ | 0.332 | 0.001 |
| 18:1n-7t | 2.8 | 0.1 | 2.9 | 0.1 | 3.3 | 0.2 | 2.6 | 0.1 | 3.0 | 0.2 | 2.9 | 0.2 | 0.985 | 0.072 |
| 18:0 | 19.8 | 0.7 | 19.9 | 0.4 | 19.2 | 0.6 | 20.7 | 0.7 | 20.7 | 0.6 | 18.9 | 0.7 | 0.621 | 0.102 |
| 20:4n-6 ARA | 0.6 | 0.1 | 0.7 | 0.1 | 0.4 | 0.1 | 0.6 | 0.1 | 0.8 | 0.1 | 0.7 | 0.1 | 0.421 | 0.725 |
| 20:5n-3 EPA | 0.5 | 0.1 | 0.4 | 0.1 | 0.5 | 0.2 | 0.4 | 0.1 | 0.4 | 0.1 | 0.4 | 0.1 | 0.096 | 0.754 |
| 20:3n-6 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.902 | 0.217 |
| 20:0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.297 | 0.494 |
| 22:5n-6 DPA-3 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.2 | 0.2 | 0.0 | 0.0 | 0.944 | 0.574 |
| 22:6n-3 DHA | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.501 | 0.782 |
| 22:5n-3 DPA-3 | 0.3 | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 | 0.3 | 0.1 | 0.4 | 0.1 | 0.3 | 0.1 | 0.673 | 0.425 |
| ∑SFA | 47.1 | 1.1 | 46.4 | 0.6 | 47.1 | 1.7 | 47.0 | 1.0 | 47.9 | 0.6 | 45.6 | 1.1 | 0.250 | 0.598 |
| ∑MUFA | 45.1 | 1.0 | 45.4 | 0.6 | 45.6 | 2.0 | 45.4 | 0.7 | 42.6 | 0.9 | 46.2 | 1.1 | 0.091 | 0.104 |
| ∑PUFA | 7.8 | 0.8 | 8.2 | 0.5 | 7.2 | 0.5 | 7.7 | 0.8 | 9.5 | 0.7 | 8.2 | 0.8 | 0.482 | 0.298 |
| ∑n-3 | 2.8 | 0.2 | 2.9 | 0.2 | 2.8 | 0.2 | 2.7 | 0.3 | 3.2 | 0.4 | 2.9 | 0.3 | 0.505 | 0.279 |
| ∑n-6 | 4.8 | 0.5 | 5.1 | 0.3 | 4.2 | 0.3 | 4.8 | 0.6 | 6.1 | 0.4 | 5.0 | 0.6 | 0.471 | 0.304 |
| ∑other FA | 5.0 | 0.6 | 4.3 | 0.2 | 4.8 | 0.3 | 4.2 | 0.1 | 3.9 | 0.3 | 5.1 | 0.7 | 0.297 | 0.330 |
| **mg/100g** | | | | | | | | | | | | | | |
| 20:4n-6 ARA | 13.5 | 2.3 | 12.5 | 2.0 | 12.7 | 4.2 | 14.5 | 2.7 | 14.3 | 4.4 | 10.9 | 2.2 | 0.620 | 0.752 |
| 20:5n-3 EPA | 11.5 | 2.2 | 8.3 | 1.9 | 13.5 | 5.5 | 10.7 | 2.4 | 8.9 | 4.1 | 7.4 | 1.9 | 0.297 | 0.571 |
| 22:5n-6 DPA-3 | 0.8 | 0.4 | 1.8 | 1.5 | 0.0 | 0.0 | 0.9 | 0.4 | 6.2 | 5.9 | 0.5 | 0.4 | 0.785 | 0.182 |
| 22:6n-3 DHA | 1.9 | 0.7 | 2.0 | 0.6 | 0.8 | 0.8 | 2.5 | 0.8 | 2.5 | 1.3 | 1.7 | 0.6 | 0.957 | 0.604 |
| 22:5n-3 DPA-3 | 5.3 | 1.8 | 6.8 | 1.7 | 3.7 | 3.7 | 7.1 | 2.2 | 9.4 | 4.1 | 4.9 | 1.7 | 0.839 | 0.626 |

[1] Means with different superscripts [A, B, C, D, E] within rows significantly differ (P<0.05).
[2] as per Table 2.

*Interactions between sire breed and sex*

Muscle EPA composition of Black Suffolk-sired ewe lambs was higher than for ewes of other sire breeds ($P$<0.012). For wethers, muscle EPA composition of Dorset-sired lambs was higher compared to wethers from other sire breeds. Muscle EPA composition of Black Suffolk- and White Suffolk-sired ewe lambs was higher than equivalent wether lambs, unlike Dorset-sired lambs (Figure 9.1a). Muscle 20:3n-6 composition of Dorset-sired wether lambs was higher than for their ewe counterparts ($P$<0.026; Figure 9.1b). Muscle 20:3n-6 of White Suffolk-sired ewe lambs was higher compared to wethers. All other interactions between sire breed and sex on analysed FA composition had no significance ($P$>0.05).

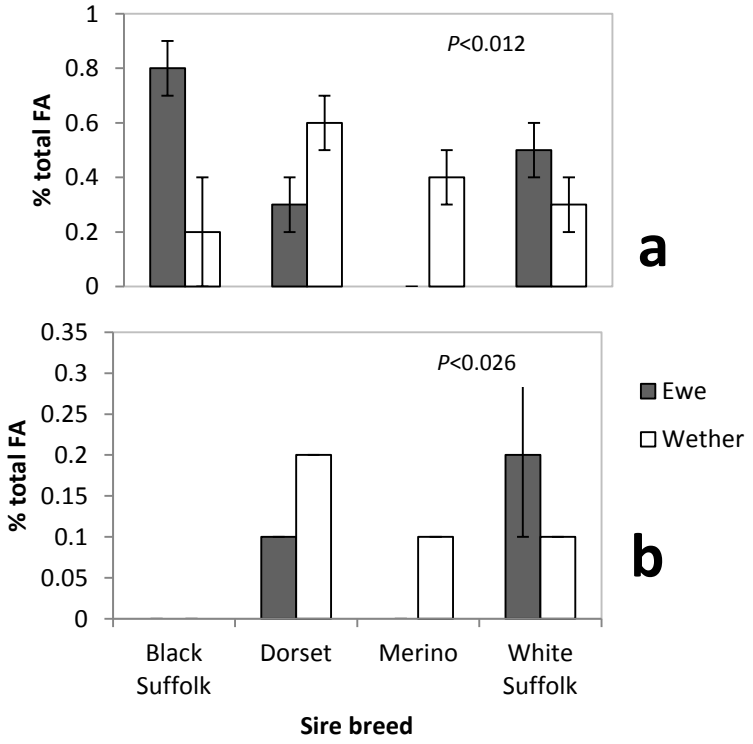


**Figure 9.1.** Sire breed and sex interaction on a) 20:5n-3 (EPA); and b) 20:3n-6, percentage total fatty acid (% total FA) composition of muscle tissue from Australian dual-purpose lambs. Other sire breed and sex interactions on individual or sum FA were found to have no significance ($P$>0.05).

*Fatty acid content as mg/100g*

FA content (mg/100g) was observed as having similar trends to composition (% total FA) in response to sex and sire breed effect. All effects had no significance ($P$>0.05), except liver DPA-3 content in Black Suffolk-sired lambs being higher compared to other sire breeds (P<0.048).

**Discussion**

In this study, FA data was transformed, for the most part, into percentage of total FA to allow samples from two feeding trials to be merged without introducing unnecessary bias. This was assisted by the randomised selection of feeding trial lambs being balanced by sire breed and sex and using almost identical management and sampling protocols to nullify many sources of error. However, some content (mg/100g) FA data was included of nutritionally significant FAs. These demonstrated similar responses to composition except regarding Black Suffolk-sired lamb liver DPA-3 content, which is thought to have arisen from Black Suffolk sire breed being excluded from one of the feeding trials.

It was demonstrated that subcutaneous adipose, muscle, kidney, heart and liver tissue differed in FA composition. This is thought to arise from differences in FA functions within the tissues and variations in tissue phospholipid concentrations (De Deposito et al., 2009; Getz *et al.*, 1968). For instance, phospholipid concentration is highest in organs such as kidneys, liver, and heart, followed by muscle, and lowest in adipose tissue (Nuernberg *et al.*, 2005). This stems from increased triacylglycerols diluting phospholipid concentrations, as phospholipids are a main constituent of cell membranes. Cell membranes are rich with unsaturated FA (Cloete *et al.*, 2007), with ruminants preferentially depositing PUFA as cell membrane phospholipids (Enser et al., 1998). Therefore, differences in phospholipid concentrations would be reflected in both absolute and relative (percent) levels of total and individual tissue PUFA. Park and Washington (1993), in their research with goats, found similar differences in PUFA composition and the absence of individual FAs between tissues as observed in

our study. Significant breed variations in the FA composition of the adipose tissue of grazing Jersey and Limousin cattle have also been previously reported (Malau-Aduli *et al.*, 1997).

Sex had a significant effect on heart and muscle tissue 22:5n-6 and 18:1n-9 FA composition, respectively. This reflects the findings from previous studies which identified sex as a causal factor influencing ruminant meat quality (Dransfield *et al.*, 1990) and FA composition (Cloete et al., 2007; Mezoszentgyorgyi et al., 2001). These studies highlighted differences in the effect of sex hormone concentrations on ruminant development as being the underlining mechanism causing FA composition differences. Similarly, this difference is thought to be the causal agent of our observed significant effect of sex on individual FA. This interpretation is reinforced by the findings of Malau-Aduli et al., (2000c) in cattle which showed that male and female FA composition varies with age. It is thought that in lambs, this is expressed due to differences in rumen biohydrogenation functionality between the sexes (De Deposito et al., 2009). However, in our study, lambs were all of the same age and having typical rumen function. Furthermore, as aforementioned, FA data was assessed as percentages of total FA. This would negate the potential effect of female ruminant's characteristically higher carcass lipid composition, or fattiness, than males at comparable liveweights (Mezoszentgyorgyi et al., 2001). The relative absence of sex effect on other individual FAs found in this study is reflected in other lamb trials (De Deposito et al., 2009; Horcada et al., 1998).

Short-chain SFA, being SFA with hydrocarbon chains under 13 carbon atoms in length (<C13), tissue composition of Merino-sired lambs was observed to be comparatively greater than the other studied sire breeds. Previously, sire breed had been found to affect ruminant meat quality (Santos-Silva et al., 2002b) and FA composition (Malau-Aduli et al., 2000c). For instance, Mezoszentgyorgyi et al., (2001) found that Booroola Merinos had more total and short-chain SFA compared to equivalent Suffolk mutton sheep. Cloete et al., (2007) observed that South African Mutton Merino muscle tissue had less total SFA than Dorper sheep. These differences may indicate variation in $\Delta^9$-desaturase and elongase activity between

sire breeds, as was found between Jersey and Limousin cattle (Malau-Aduli *et al.*, 1998).

Sire breed was shown to affect the composition of several individual FA in kidney, *Longissimus dorsi* muscle, subcutaneous adipose, heart and liver tissues. Previous research has found significant variations between sire breeds FA composition in response to dietary supplementation (Costa et al., 2009; Sinnett-Smith and, Woolliams, 1988; Wachira *et al.*, 2002). These variations indicate differences in FA metabolism, lipogenesis and deposition which stem from introduced variation in paternal genetics. However, the observed absence of sire breed effect on the summations of SFA, total PUFA, n-3 PUFA and n-6 PUFA suggests a close genetic relation between the sire breeds assessed in our study. Other studies comparing lambs of shared origin breed have found similar outcomes. For example, Santos-Silva et al., (2002b) found the FA composition of purebred Merino Branco muscle did not differ from crossbred Ile de France x Merino Branco. Likewise, L'Estrange and Hanrahan (1980) found no differences in subcutaneous adipose FA composition between pure Galway, Balway x Fingalway, and Galway x (Finn x Texel) lambs. However, contrasting findings in our study showed purebred Merino lambs had higher percentages of individual SFA than crossbred it can be deduced that sufficient genetic variation is introduced with sire breed to affect the composition of some FA.

The variations observed with individual FA in tissue may have arisen from differences in ruminant fatness and maturity size as cattle maturity size has been associated with differences in adipose tissue FA composition (Zembayashi and, Nishimura, 1996). Furthermore, lean lamb breeds have been shown to have higher muscle PUFA composition than fatter breeds, as a result of the dilution effect of neutral storage lipid (triacylglycerols) or marbling fats on phospholipid concentrations (Fisher et al., 2000). These may also explain the observed sex and sire breed interaction effect on individual FA and the higher composition of the long-chain PUFA – DHA – in the liver tissue of Dorset-sired lambs compared to other breeds.

**Conclusion**

This study found variation between tissue FA compositions, with for heart, liver and kidney tissue comparatively high total PUFA and in *Longissimus dorsi* muscle tissue total MUFA composition highlighting differences in nutritional quality. Sire breed was shown to affect the composition of several individual FA. For instance, Dorset-sired lamb liver DHA composition was the highest observed and purebred Merino contained higher specific SFA composition than crossbred counterparts. Sex also affected individual FA composition, albeit to a lesser extent than sire breed. Our findings support the hypothesis that tissue FA composition varies between lambs sire breed and sex. Consequently, Australian dual-purpose production systems must account for sex and sire breed effects on FA composition and associated nutritional and sensory qualities when designing breeding and management strategies to best achieve production goals. Furthermore, the nutritional benefits, within reason, from lamb heart, liver and kidney consumption have been highlighted.

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Chapter 10

# Effect of nutritional plane on the fatty acid profiles of heart, kidney, liver, adipose and muscle tissue of lambs

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**Abstract**

This investigation focused on the effect of plane of nutrition and its interactions with sire breed and sex on Australian dual-purpose lamb fatty acid (FA) content and concentration in the subcutaneous adipose, *Longissimus dorsi* muscle, kidney, heart and liver tissues. Tissue samples were taken from 40 lambs on different planes of nutrition (high – typical pasture grazing; and low – simulated-drought ration), sire breeds (Black Suffolk, Dorset, Merino, and White Suffolk), and sex (ewes and wethers). FA data were computed in percentage and mg/100g formats and analysed in SAS. Significant variations between tissues were detected. The highest proportions and contents of many individual FA and total FA were obtained in lambs on the high plane of nutrition with regards to polyunsaturated FA (PUFA) proportions (as % total FA) in the muscle ($P<0.001$), liver ($P<0.001$), and subcutaneous adipose ($P<0.020$) tissues. Heart and kidney tissue FA contents and concentrations were minimally influenced by plane of nutrition, while sire breed, sex and plane of nutrition interactions had limited but significant effects on some tissue FA content and concentration. Plane of nutrition was identified as a major causal factor influencing tissue FA content and concentration, hence lamb product quality. Australian dual-purpose lamb producers can optimise their plane of nutrition during periods of

abundance or drought to best achieve enhanced nutritional and sensory qualities of products associated with FA content and concentration.

(**Keywords**: *Nutritional plane, Crossbred, Fatty acid profile, Meat quality, Organ*)

**Introduction**

Understanding fatty acid (FA) content (expressed as mg/100 g) and concentration (as percent of total FA) of consumable tissues provides an objective insight into lamb product quality. This stems from the intrinsic relationship of specific FAs with sensory quality attributes, especially flavour, aroma and appearance which have been reviewed elsewhere (Wood et al., 2008). Furthermore, omega-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (n-3 LC-PUFA) - in particular eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) - have strong associations with promoting human health (Polidori *et al.*, 2011). For instance, low levels of saturated FA (SFA) and high levels of unsaturated FA, such as monounsaturated (MUFA) and PUFA, in particular n-3 LC-PUFA, have been linked with reduced cardiovascular disease risk (Kris-Etherton *et al.*, 2003).

Consistency in lamb product quality is fundamental to securing market share for Australian dual-purpose lamb producers. However, deviations are inherent with seasonal variations and production localities affecting feed availability, type and nutritional plane (Ponnampalam *et al.*, 2010). Moreover, this variability is predicted to be exacerbated with climate change (Hegarty, 2012; Nardone et al., 2010). Lamb tissue FA content and concentration have been shown to be influenced by changes in diet quality and concentration, particularly in relation to the amount and type of dietary fat (Aurousseau *et al.*, 2004; Bas and, Morand-Fehr, 2000; Velasco *et al.*, 2004). Demirel *et al.* (2004) found the PUFA content of lamb muscle tissue increased with corresponding increases in dietary PUFA. Sire breed and sex have also been shown to affect ruminant tissue FA content and concentration (Malau-Aduli *et al.*, 2000a; Malau-Aduli *et al.*, 2000b; Mezoszentgyorgyi et al., 2001). This is relevant as

dual-purpose sheep producers routinely crossbreed meat-type rams with a core purebred Merino ewe flock to introduce desirable growth, meat and wool traits to any progeny (ABARE., 2012).

To the best of our knowledge, research into the effect of diet and its interactions with sire breed and sex on FA profiles have focused only on: 1) muscle and adipose tissue, which ignores other consumable lamb products such as kidneys, liver and heart; and 2) the effect of supplementation, rather than differences in the actual basal diet or plane of nutrition. This study intends to fill this knowledge gap by investigating the effect of plane of nutrition and its interactions with sire breed and sex on the FA content and concentration of the subcutaneous adipose, muscle, kidney, liver and heart tissues in Australian dual-purpose lambs.

**Material and methods**

All experimental procedures were in accordance with the University of Tasmania (UTAS) Animal Ethics Committee guidelines, the 1993 Tasmania Animal Welfare Act and the 2004 Australian code of Practice for the Care and Use of Animals for Scientific Purposes (NH & MRC 2004). This study was conducted at the UTAS Farm, Cambridge, Hobart, Tasmania, Australia.

*Animals, experimental design and feed*

This trial included a total of 48 first filial generation crossbred and purebred Merino lambs from purebred Merino ewes mated to White Suffolk, Black Suffolk, Dorset and Merino rams in separate paddocks under the same pasture-based management at a ratio of 1 ram to 100 ewes to generate $F_1$ progeny. Lambs were weaned onto ryegrass pasture at 12 weeks old and identified using National Livestock Identification ear tags. At 6 months of age, experimental lambs were subjected to a 9-week feeding trial which included a 3-week adjustment phase and provided lambs the same protein-rich supplementation routine as described by Holman et al., (2012). Lambs had *ad libitum* access to basal diets of differing planes of nutrition (Table

10.1), with lambs fed lucerne hay (low nutritional plane) or ryegrass pasture (high nutritional plane). Lamb allocation into treatment groups was random. Sire breeds on the high nutritional plane comprised Black Suffolk, Dorset, Merino and White Suffolk; and the low nutritional plane included Dorset, Merino and White Suffolk.

*Slaughter and sampling*

At the completion of the feeding trial, all experimental lambs were slaughtered at a commercial abattoir (Gretna Quality Meats, Gretna, Tasmania, AUS), except the purebred Merino ewe lambs which were retained as replacement stock for breeding purposes. Immediately following slaughter, *Longissimus dorsi* muscle with overlaying subcutaneous adipose, kidney, liver and heart tissue samples were removed from each carcass, snap-frozen in liquid nitrogen, transported to the lab and stored at -20°C until analysed for FA.

*Lipid extraction and analysis*

Using a modified Bligh and Dyer protocol (Bligh and, Dyer, 1959), all adipose, kidney, heart, liver and muscle tissue samples were extracted overnight as a single-phase using $CHCl_3$:MeOH:$H_2O$ (1:2:0.8 v/v). The phases were separated using $CHCl_3$:saline$H_2O$ (1:1 v/v). Rotary evaporation of the lower chloroform phase provided the total lipid extract.

Aliquots of the total lipid extract were transmethylated in MeOH:$CHCl_3$:HCl (10:1:1 v/v) for 2 hrs at 80°C. Following the addition of Milli-Q H2O (1ml), FA methyl esters (FAME) were extracted three times using hexane:$CHCl_3$ (4:1 v/v) and reduced under a nitrogen stream. A known concentration of internal injection standard (19:0 FAME) was added. Samples were analysed using an Agilent Technologies 7890B gas chromatograph (GC) (Palo Alto, California USA) equipped with an Equity™-1 fused silica capillary column (15m x 0.1mm internal diameter and 0.1µm film thickness), a flame ionisation detector, a split/splitless injector and an Agilent Technologies 7683B Series autosampler. At an oven temperature of 120°C, samples were injected in

splitless mode and carried by helium gas. Oven temperature was raised to 270°C at 10°C per min, and then to 310°C at 5°C per min. Peaks were quantified using Agilent Technologies ChemStation software (Palo Alto, California USA).

FAME were identified using GC-mass spectrometric (GC/MS) analysis using a Finnigan Thermoquest GCQ GC/MS fitted with an on-column injector and Thermoquest Xcalibur software (Austin, Texas USA). The GC had a HP-5 cross-linked methyl silicone-fused silica capillary column (50m x 0.32mm internal diameter) of similar polarity to that described above. Helium was used as the carrier gas, and operating conditions were as per Miller *et al.* (2006).

**Table 10.1** Fatty acid content and concentration as percentage of total fatty acids of basal and supplementary feeds

| | Content (mg/100g) | | | | Concentration (% total FA) | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acids[1] | Pasture | Hay | Barley | *Spirulina* | Pasture | Hay | Barley | *Spirulina* |
| 14:0 | 11.1 | 0.4 | 1.3 | 0.0 | 1.9 | 0.7 | 0.3 | 0.0 |
| 15:0 | 3.3 | 1.7 | 0.3 | 0.2 | 0.4 | 2.9 | 0.1 | 0.8 |
| 16:3 | 0.0 | 0.2 | 0.1 | . | 0.0 | 0.3 | 0.0 | . |
| 16:1n7c | 4.9 | 1.0 | 1.0 | 5.6 | 0.7 | 1.7 | 0.2 | 2.2 |
| 16:0 | 166.7 | 26.3 | 103.5 | 79.6 | 24.4 | 45.6 | 25.7 | 53.2 |
| 17:1n-8c | 3.1 | 0.4 | 0.2 | 0.4 | 0.5 | 0.7 | 0.0 | 0.2 |
| 17:0 | 1.3 | 0.8 | 0.4 | 0.4 | 0.4 | 1.4 | 0.1 | 0.2 |
| 18:2n-6 LA | 44.2 | 7.4 | 196.7 | 20.5 | 6.5 | 12.8 | 48.6 | 8.0 |
| 18:3n-3 ALA | 272.1 | 3.2 | 22.1 | 1.0 | 41.3 | 5.6 | 5.6 | 3.6 |
| 18:1n-9c | 81.0 | 3.5 | 52.0 | 6.9 | 12.2 | 6.1 | 12.9 | 13.7 |
| 18:1n-7c | 6.0 | 0.8 | 4.1 | 0.8 | 0.9 | 1.5 | 1.0 | 0.3 |
| 18:0 | 45.1 | 4.5 | 6.3 | 2.5 | 6.9 | 7.9 | 1.6 | 1.6 |
| 20:5n-3 EPA | 0.6 | 0.1 | 0.6 | 0.0 | 0.1 | 0.1 | 0.2 | 0.0 |
| 20:3n-6 | 0.2 | 0.2 | 1.7 | 0.0 | 0.0 | 0.4 | 0.4 | 0.0 |
| 20:4n-3 | 0.0 | 0.5 | 3.2 | 0.0 | 0.0 | 0.9 | 0.8 | 0.0 |
| 20:2n-6 | 4.9 | 0.0 | 0.5 | 0.0 | 0.8 | 0.0 | 0.1 | 0.0 |
| 20:0 | 3.1 | 1.2 | 0.8 | 0.0 | 0.5 | 2.1 | 0.2 | 0.0 |
| 22:5n-6 DPA-6 | 0.5 | 0.0 | 1.1 | 0.0 | 0.1 | 0.0 | 0.3 | 0.0 |
| 22:4n-6 | 0.5 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 |
| 22:5n-3 DPA-3 | 1.0 | 0.0 | 0.7 | 0.0 | 0.4 | 0.0 | 0.2 | 0.0 |
| 22:1n-11c | 1.0 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| 22:1n-9c | 0.0 | 0.0 | 0.4 | . | 0.0 | 0.0 | 0.1 | . |
| 22:0 | 2.2 | 1.8 | 1.2 | 0.0 | 0.5 | 3.2 | 0.3 | 0.0 |
| 24:6n-3 | 0.1 | 1.0 | 0.6 | 0.0 | 0.0 | 1.7 | 0.1 | 0.0 |
| 24:0 | 1.0 | 1.8 | 0.6 | 0.0 | 0.2 | 3.2 | 0.2 | 0.0 |
| ∑SFA | 233.9 | 38.5 | 114.5 | 82.7 | 35.3 | 67.2 | 28.4 | 55.6 |
| ∑MUFA | 403.0 | 256.5 | 313.3 | 16.8 | 15.1 | 10.5 | 15.0 | 23.0 |
| ∑PUFA | 326.0 | 12.8 | 228.0 | 42.3 | 49.6 | 22.3 | 56.5 | 21.5 |
| ∑n-3 PUFA | 275.6 | 5.0 | 27.2 | 2.5 | 41.9 | 8.4 | 6.9 | 5.1 |
| ∑n-6 PUFA | 50.3 | 7.6 | 200.7 | 39.7 | 7.6 | 13.4 | 49.6 | 16.3 |
| ∑n-3LC-PUFA | 2.7 | 1.6 | 5.1 | 0.0 | 0.5 | 2.8 | 1.2 | 0.0 |
| ∑other FA | 320.7 | 257.0 | 261.1 | 24.1 | 1.1 | 0.8 | 0.7 | 16.5 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1n-5, 15:1n-6, 16:1n-9, 16:1n-7, Br17:1, 17:1n-8+a17:0, 17:1, 18:1n-9, 18:1n-7, 18:1n-5, 18:1, 19:1, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 22:1n-7, 24:1n-11, 24:1n-9, 24:1n-7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4n-3, 18:3n-6, 18:2n-6, 18:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 21:5n-3, 22:6n-3, 22:5n-3, 22:5n-6, 22:4n-6, 24:6n-3, 24:5n-3; ∑n-3 LC-PUFA is the sum of 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3, 21:5n-3, 24:6n-3, 24:5n-3; ∑n-3 PUFA is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:6n-3, 22:5n-3, 24:6n-3, 24:5n-3; ∑n-6 PUFA is the sum of 18:2n-6, 18:3n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6; ∑other FA is the sum of individual FA present at <0.1%:

## Statistical analysis

FA peak areas were transformed into percentage of total FA (% total FA) and content within tissue (mg/100 g) formats. These data were then independently analysed using 'Statistical Analysis System' software (SAS Institute., 2009). In both analyses, summary statistics were initially calculated and means, standard deviations, and minimum and maximum values assessed for errors and outliers. Factorial ANOVA (PROC GLM) analysis (SAS Institute., 2009) was then used, with nutritional plane (high, low), tissue (adipose, heart, kidney, liver and muscle) interactions fitted as fixed effects and FAs (14:0, 15:0, 16:1n-9c, 16:1n-7c, 16:0, 17:0, 18:2n-6 (linolenic acid; LA), 18:3n-3 (alpha-linolenic acid; ALA), 18:1n-9, 18:1n-7c, 18:1n-7t, 18:0, 20:4n-6, EPA, 20:3n-6, 20:4n-3, 20:2n-6, 20:0, DPA-6, DHA, DPA-3, 22:0, 23:0, 24:0, ∑SFA, ∑MUFA, ∑PUFA, ∑n-3 PUFA, ∑n-6 PUFA, ∑other FA) and percentage lipid in tissue (% Lipid) as dependent variables. Bonferroni's pairwise comparison tests were used to determine mean differences at $P<0.05$ level of significance.

**Results**

*Tissue fatty acid content (mg/100g)*

Adipose tissue contents (mg/100g) of 16:1n-9c, 16:1n-7c, 16:0, 17:0, 18:2n-6, ALA, 18:1n-9, 18:1n-7c, 18:1n-7t, ∑MUFA, ∑PUFA, ∑n-3 PUFA, ∑n-3 LC PUFA, and ∑other FA in lambs on high nutritional plane were greater, but lower in 20:2n-6, 20:0, DPA-6, and 22:0, than those of their counterparts on low nutritional plane (Table 2; Figure 1). A comparative observation of the heart tissue indicates that lambs on a high nutritional plane contained higher contents of 15:0, 16:1n-7c, ALA, DPA-3 and ∑n-3 PUFA than the low nutritional plane treatment group that recorded the highest 20:4n-3, 20:2n-6, 20:0 and DPA-6 (Table 10.2). Kidney tissue from lambs on high nutritional plane had greater contents of 15:0, 16:1n-7c, ALA, 18:1n-9, 18:1n-7t, 18:0, EPA, DPA-3, 22:0, 24:0, ∑SFA, ∑MUFA and ∑n-3 PUFA, but lower 20:2n-6 and DPA-6 than for the low nutritional plane samples (Table 10.2). High nutritional plane liver tissue had the greatest content of all FA, except for 14:0, 15:0, 16:1n-9c, 17:0, 20:2n-

6, DPA-6 and 23:0 for which no difference was observed between the two nutritional planes ($P$>0.05; Table 10.2; Figure 10.1). In the *Longissimus dorsi* muscle tissue, 14:0, 15:0, 16:1n-9c, 16:1n-7c, 16:0, 17:0, LA, ALA, 18:1n-9, 18:1n-7c, 18:1n-7t, 18:0, EPA, ∑SFA, ∑MUFA, ∑PUFA, ∑n-3 PUFA, ∑n-6 PUFA, and EPA+DPA-3+DHA were greatest in the high nutritional plane treatment group compared to the low nutritional plane (Table 10.2; Figure 10.2). All other effects of nutritional plane on FA content did not reach statistical significance ($P$>0.05).

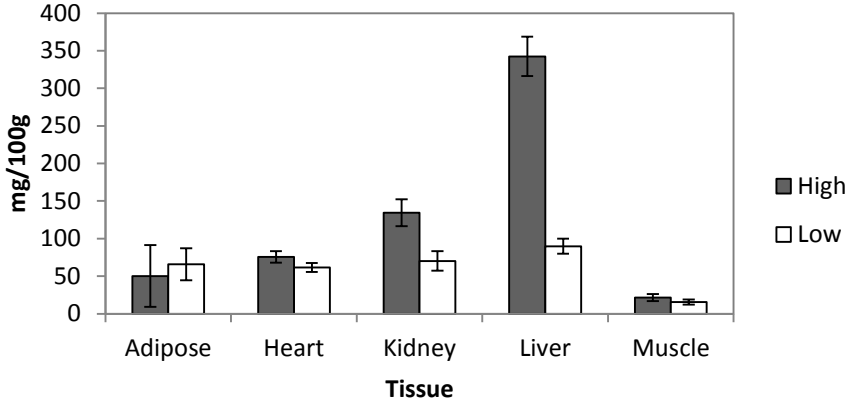


**Figure 10.1** Effect of plane of nutrition (high and low) on tissue omega-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acid content (mg/100g).

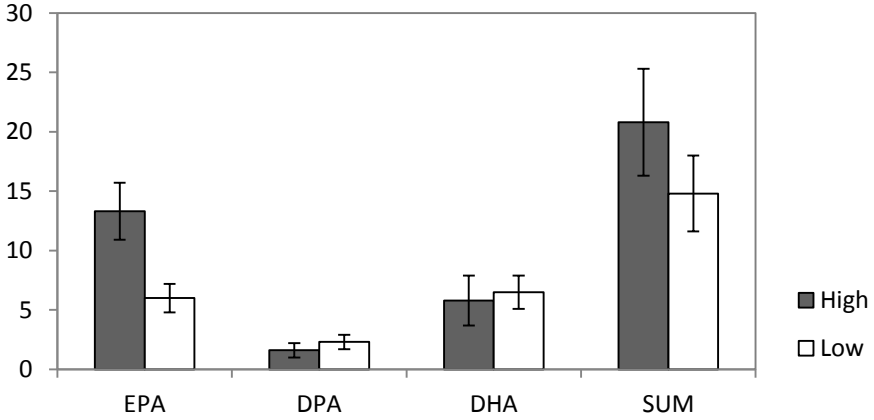


**Figure 10.2** Effect of plane of nutrition (high and low) on EPA (20:5n-3; $P$<0.009), DPA-3 (22:5n-3; P<0.403), DHA (22:6n-3; P<0.797), and their sum (EPA+DPA-3+DHA; $P$<0.291) content (mg/100g) in *Longissimus dorsi* muscle tissue.

*Tissue fatty acid concentration (as % total FA)*

For the high nutritional plane adipose tissue samples, the relative levels (as % total FA) of 16:1n-9c, 16:1n-7c, ALA, 18:1n-7c, 18:1n-7t, 20:3n-6, and 22:0, were greater than for the low nutritional plane. 20:2n-6, 20:0 and ∑SFA were higher in the low nutritional plane adipose tissue (Table 10.3). In the heart tissue from lambs with high nutritional plane, the concentration of 15:0, 16:1n-7c, ALA, 18:1n-7t, EPA, DPA-6 and ∑n-3 PUFA were higher. At low nutritional plane, heart tissue concentration of 17:0, 18:2n-6, 20:4n-3, 20:2n-6, 20:0, and ∑n-6 PUFA were greater than for the high nutritional plane (Table 10.3). At the high nutritional plane, kidney tissue concentration of 15:0, 18:1n-7t, EPA, 20:3n-6, 22:0, and ∑n-3 PUFA were greatest (% total FA). Low nutritional plane kidney tissue levels of 17:0, LA, 18:1n-7c, 20:4n-6, 20:2n-6, DPA-6, DHA and ∑n-6 PUFA were greater than for the high nutritional plane counterparts (Table 10.3). At low nutritional plane, liver tissue concentration (% total FA) of 15:0, 16:1n-9c, 16:0, 17:0, 18:1n-9, and ∑MUFA were greatest; in comparison, 18:0, EPA, 20:4n-3, DHA, 22:0, 24:0, and ∑n-3 PUFA, were highest in liver tissue at high nutritional plane (Table 10.3). *Longissimus dorsi* muscle tissue with high nutritional plane showed higher relative levels of 14:0, 15:0, ALA, and 18:1n-7t. For low nutritional plane, the concentration of LA, 20:4n-6, 20:3n-6, 20:2n-6, 20:0, DPA-6, DHA, and DPA-3 were greater than that at the high nutritional plane (Table 10.3). All other effects of nutritional plane on FA concentration had no significance ($P$>0.05).

**Table 10.2** Mean fatty acid content (mg/100g) of subcutaneous adipose, heart, kidney, liver and muscle tissue samples from lambs fed on different nutritional planes (High or Low).

| Fatty acids[1] | A[2] | | H | | K | | L | | M | | RMSE[3] | *P values*[4] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High | Low | High | Low | High | Low | High | Low | High | Low | | A | H | K | L | M |
| % lipid | 79.9 | 68.3 | 2.8 | 2.6 | 3.0 | 3.2 | 5.4 | 5.9 | 4.7 | 3.9 | 3.5 | NS | NS | NS | NS | NS |
| 14:0 | 1665.4 | 1097.5 | 13.7 | 14.1 | 1.9 | 2.4 | 11.4 | 8.4 | 71.8 | 27.8 | 6.8 | NS | NS | NS | NS | *** |
| 15:0 | 389.4 | 283.7 | 7.0 | 3.4 | 3.6 | 2.2 | 10.3 | 9.3 | 16.1 | 5.7 | 6.8 | NS | ** | * | NS | *** |
| 16:1n-9c | 220.0 | 128.6 | 4.0 | 3.3 | 2.6 | 1.5 | 11.1 | 7.9 | 9.8 | 4.1 | 8.3 | ** | NS | NS | NS | *** |
| 16:1n-7c | 774.0 | 289.8 | 7.1 | 2.0 | 3.6 | 1.7 | 17.0 | 7.8 | 38.9 | 17.9 | 7.2 | *** | *** | ** | *** | *** |
| 16:0 | 13924.0 | 9543.3 | 223.9 | 222.7 | 185.1 | 127.2 | 487.4 | 347.0 | 746.4 | 349.2 | 54.4 | * | NS | NS | * | *** |
| 17:0 | 1162.5 | 730.8 | 19.8 | 21.2 | 12.0 | 9.7 | 32.8 | 27.6 | 44.0 | 21.0 | 44.8 | * | NS | NS | NS | *** |
| 18:2n-6 LA | 945.4 | 591.0 | 214.0 | 227.7 | 87.8 | 69.0 | 171.4 | 98.0 | 113.4 | 60.8 | 32.2 | ** | NS | NS | *** | *** |
| 18:3n-3 ALA | 932.1 | 484.2 | 57.1 | 32.6 | 29.9 | 10.6 | 99.8 | 38.3 | 69.5 | 24.4 | 32.8 | *** | *** | *** | *** | *** |
| 18:1n-9 | 19894.6 | 12047.7 | 304.6 | 288.7 | 169.2 | 98.9 | 601.0 | 362.1 | 1146.3 | 552.1 | 74.5 | ** | NS | * | ** | *** |
| 18:1n-7c | 825.5 | 426.7 | 28.6 | 28.4 | 13.0 | 10.8 | 37.4 | 18.6 | 47.4 | 20.5 | 29.2 | *** | NS | NS | *** | *** |
| 18:1n-7t | 2277.6 | 1307.6 | 40.5 | 27.2 | 16.9 | 6.8 | 67.9 | 31.9 | 101.2 | 39.5 | 27.2 | ** | NS | ** | ** | *** |
| 18:0 | 13336.5 | 10020.7 | 351.4 | 318.4 | 220.6 | 126.4 | 684.7 | 317.8 | 684.2 | 315.2 | 56.4 | NS | NS | ** | *** | *** |
| 20:4n-6 ARA | 6.1 | 14.0 | 58.4 | 67.2 | 86.4 | 78.6 | 119.3 | 46.6 | 14.6 | 11.3 | 22.7 | NS | NS | NS | *** | NS |
| 20:5n-3 EPA | 9.5 | 28.6 | 43.1 | 22.6 | 68.2 | 27.8 | 119.2 | 18.0 | 13.3 | 6.0 | 11.4 | NS | *** | *** | *** | ** |
| 20:3n-6 | 0.0 | 12.0 | 7.6 | 7.9 | 6.4 | 5.0 | 14.7 | 6.5 | 2.6 | 2.6 | 2.5 | * | NS | NS | *** | NS |
| 20:4n-3 | 2.1 | 3.0 | 0.6 | 2.1 | 0.5 | 0.6 | 1.5 | 0.2 | 0.6 | 0.7 | 0.4 | NS | ** | NS | * | NS |
| 20:2n-6 | 0.0 | 53.4 | 0.1 | 1.1 | 0.2 | 0.7 | 0.2 | 0.4 | 0.1 | 0.7 | 0.3 | * | *** | ** | NS | NS |
| 20:0 | 12.7 | 64.9 | 1.6 | 3.8 | 1.9 | 1.3 | 3.3 | 1.2 | 1.3 | 2.1 | 1.2 | ** | ** | NS | ** | NS |
| 22:5n-6 DPA-6 | 3.5 | 45.9 | 0.1 | 0.7 | 0.1 | 2.1 | 0.2 | 6.9 | 0.0 | 2.8 | 0.2 | * | *** | ** | NS | NS |
| 22:6n-3 DHA | 21.9 | 7.3 | 11.6 | 13.8 | 27.9 | 23.0 | 97.6 | 41.0 | 1.6 | 2.3 | 14.5 | NS | NS | NS | *** | NS |
| 22:5n-3 DPA-3 | 9.8 | 23.5 | 19.6 | 22.3 | 36.7 | 18.7 | 120.8 | 30.5 | 5.8 | 6.5 | 3.8 | NS | NS | * | *** | NS |
| 22:0 | 1.0 | 7.5 | 3.7 | 2.7 | 13.3 | 4.7 | 7.8 | 1.2 | 0.4 | 0.4 | 0.2 | * | NS | *** | * | NS |
| 23:0 | 0.0 | 1.0 | 2.7 | 4.0 | 2.6 | 1.7 | 6.6 | 2.9 | 0.2 | 0.5 | 0.3 | NS | NS | NS | NS | NS |
| 24:0 | 1.5 | 0.4 | 2.1 | 2.3 | 9.3 | 4.6 | 8.6 | 1.8 | 0.2 | 0.4 | 0.5 | NS | NS | * | ** | NS |
| SUM | 56415.2 | 37213.0 | 1422.9 | 1340.3 | 999.4 | 635.8 | 2731.9 | 1432.0 | 3129.7 | 1474.3 | 216.8 | * | NS | * | *** | *** |
| ∑SFA | 30936.4 | 22095.4 | 640.0 | 607.2 | 458.9 | 285.8 | 1272.0 | 728.3 | 1588.8 | 732.3 | 124.9 | NS | NS | * | *** | *** |
| ∑MUFA | 26150.6 | 15309.4 | 470.4 | 429.8 | 245.7 | 143.8 | 823.0 | 470.1 | 1459.2 | 681.2 | 96.5 | ** | NS | * | *** | *** |
| ∑PUFA | 2102.6 | 1382.6 | 420.5 | 411.3 | 347.8 | 241.6 | 764.7 | 294.9 | 231.2 | 122.8 | 72.8 | * | NS | NS | *** | *** |
| ∑n-3 LC-PUFA | 50.3 | 66.0 | 75.7 | 61.5 | 134.5 | 70.3 | 342.5 | 89.8 | 21.6 | 15.7 | 11.7 | NS | NS | ** | *** | NS |
| ∑n-3 PUFA | 985.1 | 550.4 | 132.9 | 94.3 | 164.7 | 81.0 | 448.6 | 129.0 | 91.2 | 40.1 | 33.6 | ** | ** | ** | *** | *** |
| ∑n-6 PUFA | 956.3 | 719.6 | 282.4 | 307.7 | 181.7 | 156.5 | 310.8 | 162.0 | 132.4 | 78.7 | 34.0 | NS | NS | NS | *** | ** |
| ∑other FA | 2774.4 | 1574.4 | 108.0 | 108.1 | 53.0 | 35.4 | 127.8 | 61.3 | 149.5 | 62.1 | 10.3 | ** | NS | NS | *** | *** |

[1] [1] % lipid is the percentage lipid in raw tissue; SUM is the combined FA content; ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1n-5, 15:1n-6, 16:1n-9, 16:1n-7, Br17:1, 17:1n-8+a17:0, 17:1, 18:1n-9, 18:1n-7, 18:1n-5, 18:1, 19:1, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 22:1n-7, 24:1n-11, 24:1n-9, 24:1n-7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4n-3, 18:3n-6, 18:2n-6, 18:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 21:5n-3, 22:6n-3, 22:5n-3, 22:5n-6, 22:4n-6, 24:6n-3, 24:5n-3; ∑n-3 LC-PUFA is the sum of 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3, 21:5n-3, 24:6n-3, 24:5n-3; ∑n-3 PUFA is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:6n-3, 22:5n-3, 24:6n-3, 24:5n-3; ∑n-6 PUFA is the sum of 18:2n-6, 18:3n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6; ∑otherFA is the sum of individual FA present at <0.1% except ARA, DHA, EPA, and DPA.

[2] A = subcutaneous adipose tissue; H = heart tissue; K = kidney tissue; L = liver tissue; M = *Longissimus dorsi* muscle tissue.

[3] RMSE = root mean square error.

[4] NS = no significance; * = significant ($P$<0.05); ** = highly significant ($P$<0.01); *** = very highly significant ($P$<0.001). These descibe the nutritional plane and tissue type interactions.

**Table 10.3** Mean fatty acid concentration as percentage total fatty acid (% total FA) of subcutaneous adipose, heart, kidney, liver and muscle tissue samples from lambs fed on different nutritional planes (High or Low).

| Fatty acids[1] | A | | H | | K | | L | | M | | RMSE | *P values* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High | Low | High | Low | High | Low | High | Low | High | Low | | A | H | K | L | M |
| 14:0 | 2.7 | 2.6 | 0.7 | 0.8 | 0.1 | 0.2 | 0.3 | 0.5 | 2.0 | 1.4 | 1.2 | NS | NS | NS | NS | * |
| 15:0 | 0.7 | 0.6 | 0.4 | 0.3 | 0.4 | 0.2 | 0.3 | 0.6 | 0.5 | 0.4 | 0.2 | NS | *** | * | *** | ** |
| 16:1n-9c | 0.4 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 | 0.4 | 0.5 | 0.3 | 0.3 | 0.2 | * | NS | NS | * | NS |
| 16:1n-7c | 1.3 | 0.9 | 0.4 | 0.3 | 0.4 | 0.3 | 0.6 | 0.6 | 1.2 | 1.1 | 0.5 | * | * | NS | NS | NS |
| 16:0 | 23.5 | 24.3 | 14.4 | 15.0 | 18.6 | 19.2 | 17.0 | 22.6 | 22.5 | 22.4 | 4.1 | NS | NS | NS | *** | NS |
| 17:0 | 2.0 | 1.9 | 1.3 | 1.4 | 1.1 | 1.6 | 1.2 | 1.8 | 1.3 | 1.4 | 0.4 | NS | ** | *** | *** | NS |
| 18:2n-6 LA | 1.6 | 1.6 | 14.6 | 18.0 | 8.5 | 10.2 | 6.0 | 6.8 | 3.6 | 4.5 | 5.6 | NS | * | *** | NS | * |
| 18:3n-3 ALA | 1.6 | 1.3 | 3.8 | 2.6 | 3.9 | 1.4 | 3.3 | 2.7 | 2.2 | 1.8 | 2.2 | ** | *** | NS | NS | *** |
| 18:1n-9 | 33.4 | 32.3 | 19.0 | 19.2 | 16.0 | 15.2 | 20.5 | 23.9 | 35.3 | 36.6 | 8.9 | NS | NS | NS | *** | NS |
| 18:1n-7c | 1.4 | 1.1 | 1.9 | 2.0 | 1.3 | 1.7 | 1.3 | 1.3 | 1.5 | 1.5 | 0.4 | *** | NS | ** | NS | NS |
| 18:1n-7t | 4.0 | 3.2 | 2.5 | 1.7 | 1.7 | 0.9 | 2.3 | 2.1 | 3.1 | 2.6 | 1.1 | ** | *** | ** | NS | ** |
| 18:0 | 22.4 | 25.7 | 22.1 | 19.6 | 20.5 | 20.4 | 25.0 | 20.5 | 20.2 | 19.5 | 5.2 | NS | NS | NS | * | NS |
| 20:4n-6 ARA | 0.0 | 0.0 | 4.2 | 5.3 | 7.3 | 10.6 | 4.3 | 3.8 | 0.5 | 0.8 | 3.9 | NS | NS | ** | NS | ** |
| 20:5n-3 EPA | 0.0 | 0.0 | 3.2 | 1.8 | 6.3 | 3.5 | 4.2 | 1.6 | 0.5 | 0.4 | 2.3 | NS | ** | *** | *** | NS |
| 20:3n-6 | 0.0 | 0.0 | 0.5 | 0.6 | 5.1 | 0.7 | 0.5 | 0.5 | 0.1 | 0.2 | 0.3 | ** | NS | * | NS | * |
| 20:4n-3 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | NS | * | NS | * | NS |
| 20:2n-6 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | ** | *** | *** | NS | ** |
| 20:0 | 0.0 | 1.0 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 0.1 | 0.1 | *** | ** | NS | NS | ** |
| 22:5n-6 DPA-6 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 | 0.4 | 0.0 | 0.1 | 0.4 | NS | *** | *** | NS | * |
| 22:6n-3 DHA | 0.1 | 0.0 | 0.9 | 1.1 | 2.3 | 3.1 | 3.3 | 3.3 | 0.1 | 0.2 | 1.6 | NS | NS | ** | NS | * |
| 22:5n-3 DPA-3 | 0.0 | 0.0 | 1.4 | 1.6 | 3.1 | 2.6 | 4.1 | 2.3 | 0.2 | 0.4 | 1.6 | NS | NS | NS | *** | * |
| 22:0 | 0.0 | 0.0 | 0.3 | 0.2 | 1.4 | 0.9 | 0.3 | 0.1 | 0.0 | 0.0 | 0.6 | ** | NS | * | * | NS |
| 23:0 | 0.0 | 0.0 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.0 | 0.0 | 0.2 | NS | NS | NS | NS | NS |
| 24:0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.9 | 0.9 | 0.3 | 0.1 | 0.0 | 0.0 | 0.5 | NS | NS | NS | * | NS |
| ∑SFA | 52.0 | 56.0 | 40.7 | 38.8 | 44.2 | 44.8 | 45.5 | 47.1 | 47.4 | 45.9 | 7.0 | * | NS | NS | NS | NS |
| ∑MUFA | 44.3 | 40.5 | 30.0 | 29.0 | 23.3 | 21.8 | 28.1 | 31.0 | 45.3 | 45.3 | 9.7 | NS | NS | NS | * | NS |
| ∑PUFA | 3.7 | 3.5 | 29.3 | 32.2 | 32.5 | 33.4 | 26.4 | 21.9 | 7.3 | 8.8 | 13.2 | NS | NS | NS | NS | NS |
| ∑n-3 LC-PUFA | 0.1 | 0.1 | 5.6 | 4.7 | 11.9 | 9.2 | 11.8 | 7.2 | 0.7 | 1.0 | 5.2 | NS | NS | * | *** | NS |
| ∑n-3 PUFA | 1.7 | 1.4 | 9.4 | 7.3 | 15.9 | 10.7 | 15.3 | 9.9 | 2.9 | 2.8 | 5.8 | * | * | *** | *** | NS |
| ∑n-6 PUFA | 1.7 | 1.9 | 19.5 | 24.2 | 16.5 | 22.3 | 11.0 | 11.7 | 4.2 | 5.7 | 8.6 | NS | ** | *** | NS | * |
| ∑other FA | 4.8 | 3.7 | 7.3 | 7.5 | 5.1 | 5.0 | 4.4 | 3.9 | 5.0 | 4.2 | 1.9 | *** | NS | NS | * | NS |

[1] See Table 2 for abbreviations and footnotes.

*Nutritional plane interactions with sex and sire breed – effect on fatty acid concentration*

Subcutaneous adipose tissue (Figure 10.3) percent levels of ∑n-6 PUFA and LA were highest with Merino-sired lambs on the low nutritional plane, but ∑n-6 PUFA was lowest in Dorset-sired lambs on the high plane of nutrition ($P$<0.017). Other notable variations included for 17:0 which was highest in Black Suffolk-sired lambs ($P$<0.009), and 16:1n-9 in Merino-sired lambs ($P$<0.019) all on a high plane of nutrition. Palmitic acid (16:0) concentration in the liver ($P$<0.008) and kidney ($P$<0.009) was highest in Merino-sired lambs on a low nutritional plane (Figure 10.3), although the relative levels in the kidney did not differ from Merino-sired lambs on a high nutritional plane. In the heart tissue, palmitic acid was lowest in Black Suffolk-sired lambs on the high nutritional plane, highest in Merino-sired lambs on a low nutritional plane ($P$<0.002), and for the other sire breeds the plane of nutrition showed no interaction effects (Figure 10.3). In the kidney (Figure 10.3), DHA and 16:1n-7c were highest ($P$<0.001) in Dorset-sired lambs on a low plane of nutrition ($P$<0.038), 18:1n-7c was lowest in Black Suffolk-sired lambs on a high nutritional plane ($P$<0.048), while ALA was lowest in White Suffolk-sired lambs on a low plane of nutrition ($P$<0.001). No differences were observed between sire breeds receiving the high plane of nutrition. In the muscle tissue (Figure 10.3), levels of 18:1n-7t ($P$<0.040) and 18:3n-3 ($P$<0.047) were greatest in lambs on the high plane of nutrition in the following decreasing order for sire breeds: Merino, Black Suffolk and White Suffolk. In comparison, 18:1n-7c was highest in Black Suffolk-sired lambs on a high plane of nutrition ($P$<0.004), as was 17:0 ($P$<0.002).

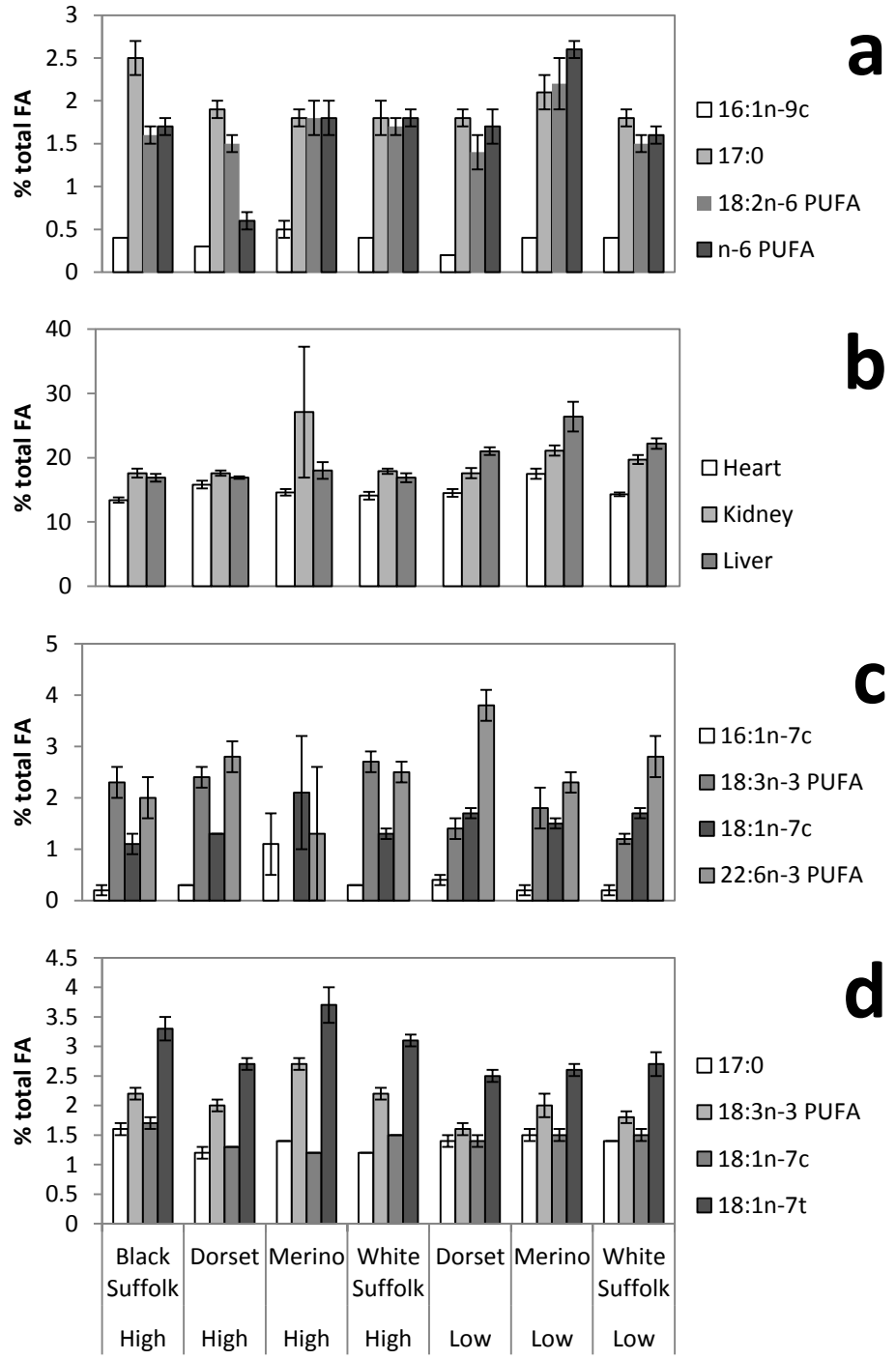


**Figure 10.3** Nutritional plane and sire breed interactions on fatty acid (FA) concentration (% total FA) of: a) subcutaneous adipose – 16:1n-9c (*P*<0.019), 17:0 (*P*<0.009), 18:2n-6 PUFA (*P*<0.027) and SUM n-6 PUFA (*P*<0.017); b) 16:0 – heart (*P*<0.002), kidney (*P*<0.009) and liver (*P*<0.008); c) kidney – 16:1n-7c (*P*<0.001), 18:3n-3 PUFA (*P*<0.001), 18:1n-7c (*P*<0.048) and 22:6n-3 PUFA (*P*<0.038); d) muscle – 17:0 (*P*<0.002), 18:3n-3 PUFA (*P*<0.047), 18:1n-7c (*P*<0.004), and 18:1n-7t (*P*<0.040).

The highest proportion of DPA was in the heart tissue of ewes on a low plane of nutrition ($P$<0.004; Figure 10.4). No other individual FA or group of FA was significantly affected by sire breed or sex interactions with plane of nutrition ($P$>0.05).

*Nutritional plane interactions with sex and sire breed – effect on lipid and fatty acid content*

Heart tissue % lipid was highest in ewes on the high plane of nutrition ($P$<0.046; Figure 10.4). Similarly, heart tissue % lipid was highest in Merino-sired lambs, regardless of plane of nutrition ($P$<0.038). Liver tissue % lipid was highest in Dorset- and Merino-sired lambs on the high nutritional plane ($P$<0.001; Figure 10.5). For kidney tissue (Figure 10.4), EPA ($P$<0.013), 20:0 ($P$<0.036), 22:0 ($P$<0.009), and 24:0 ($P$<0.012) all shared the same trend of highest content in ewes on a high plane of nutrition, and the lowest content in wethers irrespective of nutritional plane. Liver tissue 20:4n-3 content was highest in ewes on a low plane of nutrition ($P$<0.044; Figure 10.4). Highest kidney ALA content was observed in Merino-sired lambs on a high nutritional plane ($P$<0.001; Figure 10.5). No other individual FA or groups of FA or % lipid were significantly affected by sire breed or sex interactions with plane of nutrition ($P$>0.05).

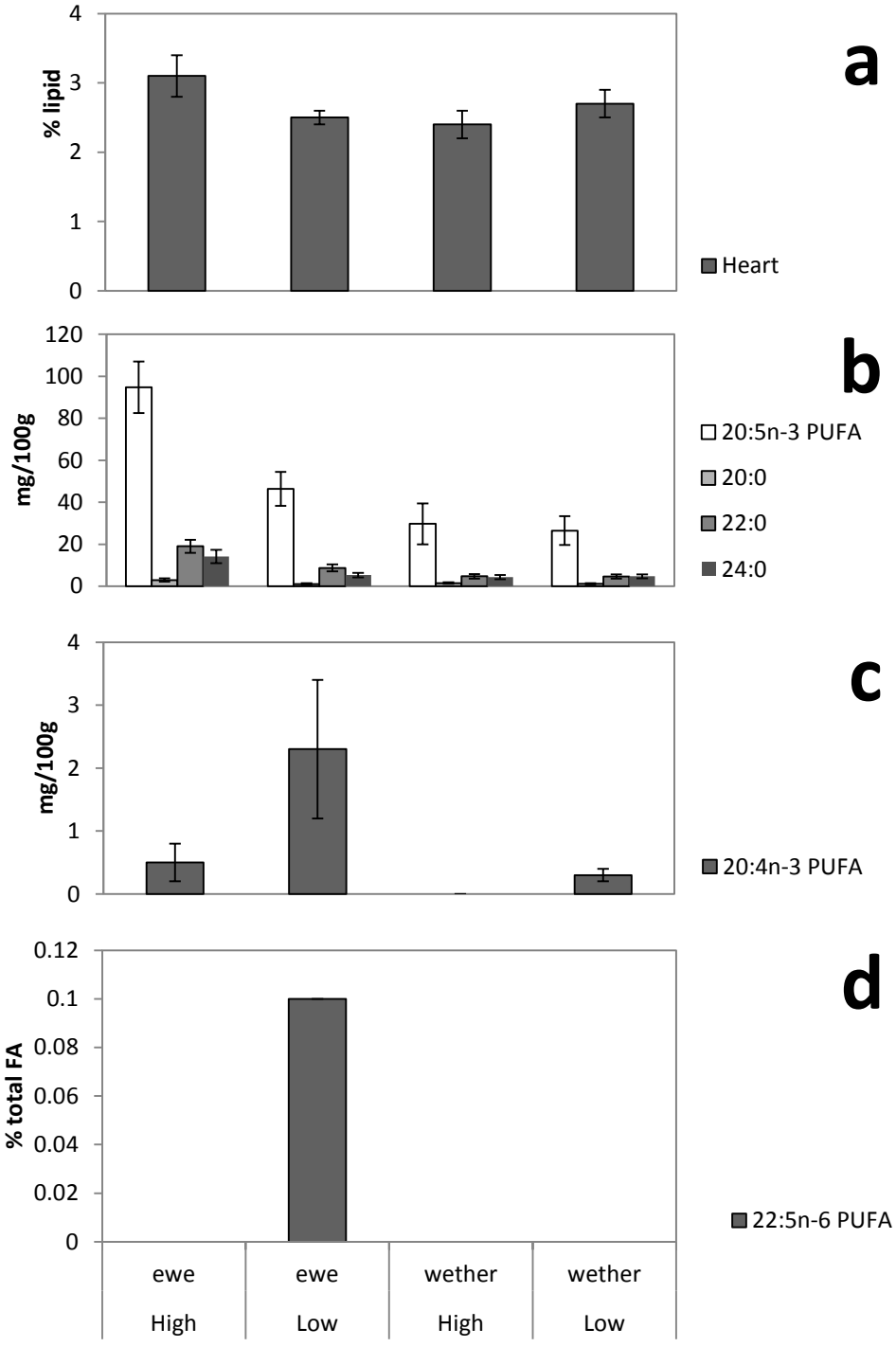


**Figure 10.4** Nutritional plane and sire breed interactions on: a) heart (*P*<0.046) lipid content (as % wet weight) and fatty acid content (mg/100 g); b) kidney – 20:5n-3 PUFA (*P*<0.013), 20:0 (*P*<0.036), 22:0 (*P*<0.009), 24:0 (*P*<0.012); c) liver (*P*<0.044); and d) heart (*P*<0.004).

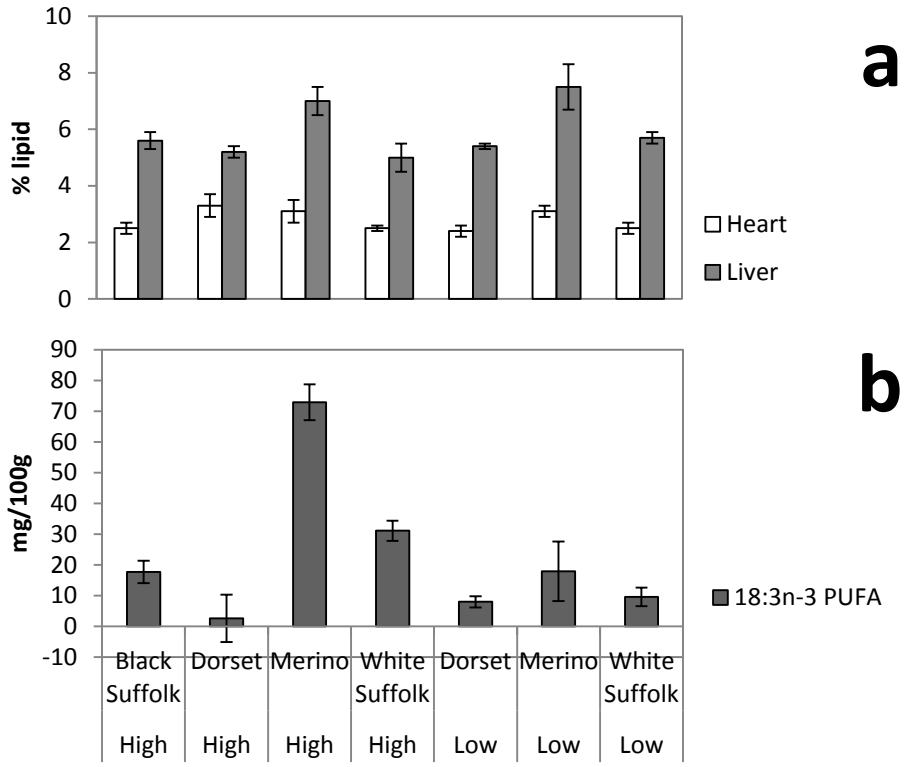


**Figure 10.5** Nutritional plane and sire breed interactions on: a) heart (*P*<0.038) and liver (*P*<0.001) lipid content (as % wet weight) and b) kidney fatty acid content (*P*<0.001).

**Discussion**

In this study, both content (mg/100 g) and concentration (as % of total FA) aspects of tissue FA profile were assessed to provide a more complete insight into FA responses to plane of nutrition. Furthermore, presenting content as mg/100 g improves interpretation of results from a human nutrition context for application to whole lamb and specific lamb tissue products.

Lamb tissue FA content was shown to correspond to plane of nutrition, with higher FA contents observed with high plane of nutrition, and the opposite found with the low nutritional plane. These observations are in agreement with several previous reports (Cooper et al., 2004; French et al., 2000; Wood et al., 2008). Ovine feeding trials have shown that diets with high FA content result in elevated tissue FA content,

primarily in the subcutaneous adipose (Cooper et al., 2004) and muscle tissues (Ponnampalam et al., 2002). A similar outcome has been observed in goats where Ryan *et al.* (2007) found Boer goats fed concentrates (high nutritional plane) had greater total FA, SFA and MUFA contents within the muscle tissue compared with their pasture-fed (low nutritional plane) counterparts.

Sheep n-3 PUFA content can be improved though increased dietary intake of ALA (Raes et al., 2004; Wachira et al., 2002). This stems from the function of ALA as a precursor to n-3 LC-PUFA, including both EPA and DHA which are desirable within human diets (Ponnampalam et al., 2010). ALA-rich pasture diets (Ponnampalam et al., 2010) generally prompt a shift in rumen FA biohydrogenation, so much so that more ALA reaches the duodenum in an unesterified form (Casey *et al.*, 1988). Hence, more ALA is available for uptake and contributes to greater FA content, especially n-3 PUFA and n-3 LC-PUFA. The high plane of nutrition used in this study was pasture-based and therefore high in ALA (41.3% of total FA or 272.1 mg/100g). It is reasonable to assume that this likely contributed to the observed concentrations of specific individual FA and tissue total n-3 PUFA. Kitessa *et al.* (2010) reported that pasture finished lamb muscle tissue had elevated n-3 PUFA content. Scerra *et al.* (2007) also showed that pasture-fed lamb muscle tissue had DPA, EPA and other n-3 PUFA contents that were greater than in stall-fed lambs. Our results further confirm these findings for a range of Australian dual-purpose lamb crossbreeds on both high and low nutritional planes.

As with FA content, tissue FA concentration (as % total FA) was observed to vary with plane of nutrition. For instance, several muscle tissue FA proportions were highest with low nutritional plane, whereas the opposite was found with subcutaneous adipose tissue. These outcomes are thought to have arisen from differences in preferential fat deposition between tissues, with UFA and essential FA preferentially deposited in muscle fat rather than in subcutaneous adipose tissue (Bas and, Morand-Fehr, 2000; Scollan et al., 2001; Wood et al., 2008). Consequently, as the different planes of nutrition provided experimental lambs with different levels of dietary FA, this would be reflected in tissue FA concentration due to the

preferential deposition. This is reinforced by the findings of Bas and Morand-Fehr (2000) in their study of the FA concentration of different tissues in lambs fed alfalfa, soybean, cotton and fish meal. Furthermore, Ponnampalam *et al.* (2010) compared grain- and pasture-fed lamb muscle tissue and found that grain-fed (high nutrition plane) lambs had short-chain FA, and DPA and DHA proportions that were higher than their pasture-fed (low nutritional plane) counterparts.

Our study found subcutaneous adipose content of most FA(s) to be highest with low plane of nutrition. Casey *et al.* (1988) observed similar findings with sheep fed a high concentrate ration (high plane of nutrition), wherein subcutaneous adipose tissue had slightly greater UFA compared to their low nutritional plane equivalents. These findings suggest individual FA concentration increases can occur within the adipose tissue during periods of nutrient stress. This indicates a loss in FA variety occurs with nutrient stress opposed to an indiscriminate 'across the board' FA decline.

Little or no difference in FA concentration or content was observed in kidney and heart tissue as far as the effect of nutritional plane was concerned. This reflects findings from previous feeding trials where only minor variation was observed in bovine kidney FA concentration when fed several feed types (Rule *et al.*, 1994); lipid supplementation had only minimal effect on rat heart tissue FA content (Charnock *et al.*, 1984). These findings all suggest the lack of change in FA content results from the preferential modification of existing lipids rather than lipogenesis (Charnock et al., 1984). Alternately, longer feeding periods and/or different basal diet management may be required to better facilitate changes to these more biochemically stable (in terms of their FA profiles) tissues.

Park and Washington (1993) found that goat kidney, heart and liver tissues share common FA characteristics and trends. This conclusion is in agreement with our findings, however, in our study, liver LC-PUFA concentration increased when lambs were on a high plane of nutrition. This difference is thought to stem from the association of liver FA concentration and free FAs within the plasma (Rule et al., 1994). Therefore, the effect of plane of nutrition on plasma FA concentration would

be more reflected within liver tissue. Rule *et al.* (1994) reaffirms this aspect with bovine liver FA content which was shown to vary with feed supplementation type.

Independently, sire breed has been shown to significantly affect ruminant tissue FA content and concentration. Generally, these differences were attributed to sire breed variation in FA regulating gene expression and enzymatic functions in FA synthesis, desaturation and elongation (Malau-Aduli *et al.*, 2011; Muchenje *et al.*, 2009). We found only minor differences between sire breeds when interacting with plane of nutrition. This is thought to arise from the close genetic proximity or relationship between the sire breeds assessed in our study. The $F_1$ lamb progeny in the current study all shared the same maternal Merino genetics. It is proposed that the genes and enzymes regulating FA synthesis do not differ greatly between these sire breeds.

Differences in tissue FA with sex are thought to be primarily due to the faster rate of fat deposition in female ruminants as demonstrated by their lipid-rich carcasses (Mezoszentgyorgyi et al., 2001). The significant interactions between sex and plane of nutrition in our study are thought to be based on this principle, as differences were only evident in tissue FA content and not concentration. Similar findings have been presented in previous research by Kitessa *et al.* (2010) who reported that muscle FA content did not differ between lamb sexes in response to supplementation. Solomon *et al.* (1992) also found that lamb sexes only varied in MUFA in response to dietary palm oil supplementation.

**Conclusion**

We identified variation in FA content and concentration in Australian dual-purpose lamb kidney, liver, heart, muscle and subcutaneous adipose tissues attributable to differences in plane of nutrition. High plane of nutrition increased FA content in all tissues assessed and comparatively elevated the proportion of n-3 PUFA, including the health-benefitting n-3 LC-PUFA – EPA, DPA and DHA – in muscle and adipose tissues compared to the low nutritional plane. The low plane of nutrition was

associated with higher proportions of several individual FA within the muscle and short-chain SFA in the liver. Heart and kidney tissue fatty acid content and concentration were generally not affected by plane of nutrition. Sire breed and sex interacted with plane of nutrition to affect FA content and concentration. These findings highlight the important role of nutrition in achieving particular targets for FA profiles and the extent of this effect in various tissue types within a lamb. In addition, our findings draw attention to the impact of drought, as is occurring more frequently due to the effects of climate change (Nardone et al., 2010; Steffen *et al.*, 2011), wherein product quality is lowered in Australian dual-purpose lambs.

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Chapter 11

# General conclusions

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The study presented in this thesis tested the overarching hypothesis that *Spirulina* supplementation would both independently and interact with sire breed, sex and plane of nutrition (or basal diet) to effect Australian dual-purpose lamb productivity and product quality. To best investigate this hypothesis, several initial questions were posed – in the **introduction** – which gave rise to subordinate hypothesis that contribute to the overall comprehension. These questioned the effect of *Spirulina* supplementation and its aforementioned interactions effects on:

1. Wool quality
2. Lamb productivity
3. Meat quality

Secondary objectives exploring queries regarding the independent effects of sire breed, sex and plane of nutrition (basal diet) on these same questioned parameters were simultaneously investigated.

To answer 'what is the effect of *Spirulina* supplementation on wool quality' we investigated physical wool traits. In **Chapter 3** we found all physical wool quality to

not be independently effected by *Spirulina* supplementation, even with MEDIUM or HIGH levels and under typical pasture-fed basal diets. This posed the questions, would parallel results be observed under simulated-drought conditions and with a LOW supplementation level? This was tested in **Chapter 2**. Similarly, only fibre diameter coefficient of variation and standard deviation physical wool traits were affected, and then only interacting with sire breed. Physical wool traits were observed to vary with sire breed, sex and basal diet, with; purebred Merino lambs' wool quality was of superior quality to crossbred counterparts; wethers having finer wool; and, clean fleece yield being lesser with typical pasture-fed basal diets.

Several indicative parameters of productivity were assessed to answer the question 'what is the effect of *Spirulina* supplementation on lamb productivity?' In **Chapter 5** we found MEDIUM *Spirulina* supplementation levels improved liveweight and some body conformation measurements. Lamb body condition score was also shown to increase as *Spirulina* supplementation level improved – with HIGH levels resulting in greatest score. However, following this initial study questions were posed. How would LOW *Spirulina* supplementation level influence these findings? Would simulated-drought basal diet (low nutritional plane) increase response sensitivity? And, will presenting data as change over feeding trial period present an alternative outcome? These were tested in **Chapter 4**. It was shown that MEDIUM and HIGH *Spirulina* supplementation levels improved both liveweight and average daily liveweight gains (ADG). These responses were most prevalent when lambs were maintained on simulated-drought basal diets. Simulated-drought diets resulted in lambs with overall smaller body conformation traits, liveweights and ADG than those with typical pasture-fed basal diets. Black Suffolk-sired lambs were largest of all sire breeds and wethers outperformed their ewe counterparts.

Lamb haematological metabolite concentrations can also be analysed to provide insight into productivity. This allowed us to ask 'will *Spirulina* supplementation be reflected in haematological metabolites?' Many of these metabolites can also aid in quantifying lamb welfare and health. Consequently, we queried whether *Spirulina* supplementation would have a positive or detrimental influence on lambs' holistic

welfare. These questions were the focus of **Chapter 6**. MEDIUM *Spirulina* supplementation levels were reflected with highest creatinine concentrations, and gamma-glytamyl transferase (GGT) concentrations increased incrementally with supplementation level. As both these metabolites indicate muscularity and growth they correspond to the findings of Chapter 4. *Spirulina* supplementation level was also observed as interacting significantly with sire breed on glucose, aspartate aminotransferase and magnesium concentrations, and with sex on albumin and globulin ratios, creatinine and GGT concentrations. Both sire breed and sex also had independent effect on haematological metabolites. Nonetheless, we were able to conclude that *Spirulina* supplementation had no detrimental effects on lamb health and welfare while prompting increases in productivity.

Lamb productivity is essentially a function of lamb feed intake. These caused us to question 'is *Spirulina* having an effect on lamb basal diet intake level?' **Chapter 7** showed that unlike sire breed neither *Spirulina* supplementation nor sex had independent effects on feed intake as determined using any of the seven models applied. *Spirulina* supplementation level did interact with sire breed as MEDIUM levels resulted in crossbred lambs specific growth rate to be highest (corroborating previous findings, Chapters 4 and 5). Female Merino lambs standardised daily feed intake (SDFI) and daily feed intake (DFI) were higher than wethers, as crossbred lambs were comparatively higher than investigated purebred Merinos. Feed intake models level of correlation varied in significance, highlighting the absence of standardisation and requirement for development in lamb feed intake modelling. These findings permit us to conclude that *Spirulina* supplementation effect on productivity does not arise through inducing greater feed intake.

Meat quality makes significant contributions to the income of Australian dual-purpose lamb producers. Consequently, answering the question of the effect of *Spirulina* supplementation on meat quality is of high importance. **Chapter 8** aimed to answer this question by assessing *Longissimus dorsi* intramuscular fat percentages (IMF) and subcutaneous adipose fat melting points (FMP) as their levels directly reflect meat quality. *Spirulina* supplementation level was shown to have no

independent effect on IMF, yet interacted with basal diet (plane of nutrition) resulting in a positive and negative association between IMF and *Spirulina* supplementation level with simulated-drought and typical pasture-fed basal diets respectively. FMP indicated greater saturated fatty acid (SFA) content with HIGH *Spirulina* supplementation levels. These suggest *Spirulina* supplementation improves meat eating quality only under simulated-drought basal diets and decreases meats nutritional quality. However, more refined investigation on meat and other lamb tissues fatty acid (FA) responses to *Spirulina* supplementation would offer better conclusions. This, as mentioned in the Introduction, is outside the scope of this thesis and is instead presented in A. Kashani's companion PhD thesis. This study, instead, questions the independent effects of sire breed, sex and basal diet (plane of nutrition) on fatty acids (FA) to indicate associated sensory and nutritional meat qualities.

FA analysis can provide insight into both associated sensory and nutritional qualities. This prompted the question 'how do sire breed and sex independently affect lamb FA profile?' This was tested in **Chapter 9** wherein five tissue types were assessed, being subcutaneous adipose, *Longissimus dorsi* muscle, kidney, heart and liver. It was found that FA composition varied significantly between tissue types and was influenced by sire breed and sex, albeit only heart and muscle tissue FAs were affected by sex. Purebred Merino tissues were shown to have highest content of many short chain SFA (<C18), and liver from Dorset-sired lambs had highest DHA composition compared to other sire breeds. Interestingly, long chain FA (>C18) compositions were not affected by sire breed. This presented the questions to whether basal diet (plane of nutrition) would affect FA profiles, and would FA content and composition share similar response trends? **Chapter 10** aimed to answer these queries.

It was found that lamb under high planes of nutrition (typical pasture-fed basal diet) had higher FA content of the majority of individual FAs than low nutritional plane (simulated-drought) counterparts. This was most obvious within liver and short chain FA within *Longissimus dorsi* muscle tissues, especially as heart and kidney FA content

showed minimal variation. FA composition showed a concentration in long chain polyunsaturated FA (PUFA) in tissues from lambs under high plane of nutrition. And, SFA composition was greatest with low plane of nutrition, especially short chain SFA in liver and adipose tissue. These findings suggested FA content and composition to provide different interpretations of the same raw information. Moreover, these allow us to conclude that sire breed, sex and plane of nutrition can be managed and matched to best enhance lamb products nutritional and sensory qualities which are associated with FA profile.

Together, these findings present *Spirulina* as an apt protein-rich supplement for Australian dual-purpose lamb producers as it improves productivity and meat quality without compromising wool quality as is inherent with many traditional supplement types. However, at present, *Spirulina* use is limited by its practicality. *Spirulina* is considerable more expensive than the majority of other protein-rich supplements available to producers in the small quantities required to achieve results. Yet, over recent years there have been exponential improvements in large-scale commercial microalgae and cyanobacterium production. This is resulting primarily from increased focus on biofuel production (Mata et al., 2010), use as a 'superfood' in human diets and comprehension of their usefulness (Becker, 2007) with many traditional feeds and resources proving finite (Hegarty, 2012; Holman and, Malau-Aduli, 2012b). Consequently, we assume *Spirulina* to steady decrease into the foreseeable future and allow its commercially viable use in lamb production not to solely rely on producers 'economies-of-scale'.

Independent to *Spirulina*, it was shown that sire breed, sex and basal diet management is imperative to optimise lamb productivity and product quality. This statement is based on the variations in wool quality traits, meat FA profile and lamb productivity observed in response to these effects.

Nevertheless, the findings of this thesis will currently aid Australian dual-purpose lamb producers and interested animal scientists when:

- Identifying an alternative protein-rich feed supplements which does not negate lamb wool quality and allows wool market interests to be exploited.
- Supplementing *Spirulina* at a cost-effective level to capitalise on optimal lamb liveweights, growth and body conformation, during various basal diets and nutritional planes.
- Tailor flock genetics (sire breeds and sexes) to achieve desired productivity and product quality goals, even under varied planes of nutrition.
- Reassuring producers that *Spirulina* supplementation does not negativity affect lamb health or productivity, as indicated via haematological metabolite concentrations.
- Allowing producers to match and manage available feed resources with sire breeds, *Spirulina* supplementation levels, and sex.
- Managing *Spirulina* supplementation to suit IMF and SFA/UFA associated sensory and nutrition qualities, respectively.
- Understanding FA content and composition associated sensory and nutritional qualities response to sire breed, sex and plane of nutrition effects and tissues in which these are observed.

Furthermore, during these studies and the construction of this thesis it became apparent of several paucities in published knowledge which would prove complementary. These could involve investigating the effect of *Spirulina* supplementation on lamb nutrient use efficiencies and partitioning between internal protein sinks. Using different sire breeds which are significant in other countries which use protein-rich supplements when producing lambs would also increase *Spirulina*'s scope of usefulness. Similarly, understanding the effects of *Spirulina* supplementation of FA gene expression and direct influence on FA profile would contribute another dimension to this thesis. Even investigating the effect of *Spirulina* on lamb endocrinological pathways would better permit comprehension of observed growth and productivity responses. Yet, as true with many studies, often more questions are raised than answered.

As a conclusion, it was evident that *Spirulina* supplementation both independently and interacts with sire breed, sex and nutritional plane to affect productivity and product quality in Australian dual-purpose lambs. Likewise, sire breed, sex and plane of nutrition (basal diet) were shown to affect these same qualities independent to *Spirulina* supplementation. Hence, the overarching hypothesis that *Spirulina* supplementation will change purebred and crossbred Merino lambs' productivity and product quality to varying extents dependent on sire breed, sex and plane of nutrition can be accepted.

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**Appendix 1**

# A Review of Sheep Wool Quality Traits

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**Appendix 2**

# *Spirulina* as a livestock supplement and animal feed

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*Appendix 2*

# *Spirulina* as a livestock supplement and animal feed

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**ABSTRACT**

*Spirulina* (*Athrospira sp*.) is an edible cyanobacterium and is a highly nutritious future feed source for many agriculturally significant animal species. Research findings have associated *Spirulina* to improvements in animal growth, fertility, and aesthetic and nutritional product quality. *Spirulina* intake has also been linked to an improvement in animal health and welfare. Its influence over animal development stems from its nutritive and protein-rich composition, thus leading to an increased commercial production to meet consumer demand. Consequently, *Spirulina* is emerging as a cost-effective means of improving animal productivity for a sustainable and viable food security future. However, our present knowledge of animal response to dietary *Spirulina* supplementation is relatively scanty and largely unknown. Therefore, the primary objective of this paper was to review past and current findings on the utilisation of *Spirulina* as a feed supplement and its impact on animal productivity and health. Only animals deemed to be of agricultural significance were investigated, hence only ruminants, poultry, swine and rabbits and their responses to dietary *Spirulina* supplementation are covered.

(**Keywords**: *swine, sheep, milk, eggs, meat quality, growth*)

## Introduction

Demand for animal products is increasing. This is due to global changes in consumer tastes and expanding markets; particularly in developing countries where affluence is spreading (Hopkins et al., 2007c; Myers and, Kent, 2003). However, two key obstacles must be overcome before this projected demand can be met; 1) increased competition for land, with urban sprawl, biofuel production and other agricultural applications using land otherwise used for animal production (Godfray et al., 2010; Poppi and, McLennan, 2010; Smith et al., 2010); and 2) climate change negatively effecting water and animal feed availability in current production regions (Gaunt et al., 2010; Poppi and, McLennan, 2010). Fortunately, innovations in animal nutrition can allow these obstacles to be avoided.

The identification of new feed resources is crucial for continued animal production viability into the future. Ideally, the new feed resource would have high nutritive value and conversion efficiency, optimise animal product qualities, and use land and water efficiently (Godfray et al., 2010; Poppi and, McLennan, 2010). Consequently, *Spirulina* is emerging as a potential candidate to fulfil these criteria. Already, *Spirulina* has been trialled in feed rations of several animal species, namely; chickens, pigs, ruminants and rabbits. It is our objective to integrate the findings from these trials into this review paper to highlight the effect of dietary *Spirulina* on animal health and productivity. We also examined *Spirulina's* history and nutrient composition.

## *Spirulina*

### *Background*

*Spirulina* (*Arthrospira* sp.) is an edible filamentous, spiral shaped cyanobacterium, formally classified as a blue-green microalga (Becker, 2007; Gouveia et al., 2008; Gupta et al., 2008). It is naturally found in the alkaline lakes of Mexico and Africa (Belay et al., 1996; Shimamatsu, 2004), where it has a long history as a food source

for their ancient human inhabitants. *Spirulina* was 'rediscovered' relatively recently by Leonard and Compere in the 1960s (Shimamatsu, 2004), and has since become a mass produced product (Shimamatsu, 2004; Spolaore *et al.*, 2006). Presently, *Spirulina* is commercially produced world-wide (Table A2.1), and is used as nutritional feed supplements for both humans and animals (Muhling et al., 2005). Although, Spolaore *et al*. (2006) suggests that approximately half total *Spirulina* production is used in animal and fish feeds.

**Table A2.1** Some of the commercial producers of *Spirulina* and their global locations[1]

| Name of Company | Location |
|---|---|
| Earthrise Farms | Calipatria, California (USA) |
| Cyanotech Corporation | Kailua Kona, Hawaii (USA) |
| Myanma Microalgae Biotechnology Project | Yangon, (Myanmar) |
| Hainan DIC Microalgae Co. Ltd. | Hainan (China) |
| Nao Pao Resins Chemical Co. Ltd. | Tainan, Taiwan (China) |
| Solarium Biotechnology | La Huayca (Chile) |
| Far East Biotechnology Co. Ltd. | Pig-Tung County, Taiwan (China) |
| DIC LIFETEC Co. Ltd. | (Japan) |
| Neotech Food Co. Ltd. | Banpong, Rajburi (Thailand) |
| Siam Algae Co. Ltd. | Bangsaothong (Thailand) |
| Ballarpur Industries Ltd. | Nanjangud, Mysore District (India) |
| TAAU Australia | Darwin, Northern Territory (Australia) |
| Sosa Texcoco | Lake Texcoco (Mexico) |
| Hills-Koor Algae Production | Elat (Israel) |

[1] Adapted from Habib et al. (2008), Ciferri and Tiboni (1985), and Sanchez et al. (2003)

*Spirulina* is produced commercially within a nutrient-rich liquid medium (Shimamatsu, 2004). Hence, it can be produced with high land-use efficiency. For instance, *Spirulina* out yields many other traditional animal feed-types, including wheat, corn, barley and soybeans, in protein output per land unit (Dismukes et al., 2008; Kulpys et al., 2009). Furthermore, *Spirulina* can be actively produced using desalinator wastewater (Volkmann et al., 2008) and using animal faecal wastes to enrich the growth medium. This has been trialled using pig (Chaiklahan et al., 2010) and cattle (Mitchell and, Richmond, 1988) faecal waste and the resultant *Spirulina* is safe to be fed back to livestock. These processes are described in more detail by Hasdai et al. (1981) and Chaiklahan et al. (2010). Nonetheless, this highlights *Spirulina's* capacity to cost-effectively treat wastes and rescue otherwise lost nutrients (Saxena et al., 1983).

Currently, *Spirulina* is relatively expensive to produce and purchase comparative to other animal feeds. This makes its use impractical in many animal production operations. Additionally, *Spirulina's* palatability, dried powdery form, and smell also proves a limitation to its use in animal production (Becker, 2007). However, *Spirulina's* production cost can be lowered with developments into a lower cost growth medium and improving operational management of *Spirulina's* nutrient use efficiency and growth rate (Peiretti and, Meineri, 2011; Raoof et al., 2006; Shimamatsu, 2004). Furthermore, research into *Spirulina* deliver methods and impact on product quality is increasingly allowing us a greater understanding of the practicalities for its use.

*Nutritional value*

*Spirulina* is nutrient-rich (Table 2). It contains all essential amino acids, vitamins and minerals. It also is a rich source of carotenoids and fatty acids, especially γ-linolenic acid (GLA) which infers health benefits (Howe et al., 2006). However, *Spirulina's* high protein content distinguishes it as a new animal feed (Belay et al., 1993; Doreau et al., 2010).

*Spirulina's* nutritional value has been the topic of several reviews (Belay et al., 1993; Ciferri, 1983; Diraman et al., 2009). Yet, its nutritional values are known to slightly vary depending on the system in which they are produced. These differences have also been the topic on several studies (Babadzhanov et al., 2004; Mata et al., 2010; Muhling et al., 2005; Tokusoglu and, Unal, 2003; Vonshak and, Richmond, 1988).

**Table A2.2** A summary of *Spirulina's* chemical and nutritional composition[1]

| | Amount | Unit |
|---|---|---|
| *Proximates* | | |
| Moisture | 4 - 9 | %DM |
| Fat (Mojonnier extraction) | 4 - 16 | %DM |
| Protein (N x 6.25) | 60 - 70 | %DM |
| Ash | 3 - 11 | %DM |
| Carbohydrates (total) | 14-19 | %DM |
| Energy | 1504.0 | kJ/100g |
| Crude Fibre | 3 - 7 | %DM |
| Lipid | | |
| *Minerals* | | |
| Calcium | 1200 | mg/kg |
| Magnesium | 3300 | mg/kg |
| Phosphate | 13000 | mg/kg |
| Potassium | 26000 | mg/kg |
| Sodium | 22000 | mg/kg |
| *Fatty Acids* | | |
| Palmitic (16:0) | 25.8 - 44.9 | % of total fatty acids |
| Palmitoleic (16:1 omega-6) | 2.3 - 3.8 | % of total fatty acids |
| Stearic (18:0) | 1.7 - 2.2 | % of total fatty acids |
| Oleic (18:1 omega-6) | 10.1 - 16.6 | % of total fatty acids |
| Linoleic (18:2 omega-6) | 11.1 - 12.0 | % of total fatty acids |
| Gamma-linolenic (18:3 omega-6) | 17.1 - 40.1 | % of total fatty acids |
| *Vitamins / Carotenoids* | | |
| β-carotene | 140000 | µg/100g |
| Total Carotenoids | 1700 | mg/kg |
| Provitamin A | 2330000 | IU $kg^{-1}$ |
| Thiamin (B1) | 34 - 50 | mg/kg |
| B2 | 30 - 46 | mg/kg |
| Niacin (B3) | 130 - 150 | mg/kg |
| B6 | 5 - 8 | mg/kg |
| B12 | 1.5 - 2.0 | mg/kg |
| Foliate | 0.50 | mg/kg |
| *Amino Acids* | | |
| Lysine | 2.60 - 4.63 | %DM |
| Phenylalanine | 2.60 - 4.10 | %DM |
| Tyrosine | 2.60 - 3.42 | %DM |
| Leucine | 5.90 - 8.37 | %DM |
| Methionine | 1.30 - 2.75 | %DM |
| Glutamic acid | 7.04 - 7.30 | %DM |
| Aspartic acid | 5.20 - 6.00 | %DM |

[1] Adapted from Habib et al. (2008), Buddhadasa and Adorno (2004), Sanchez et al. (2003), Pascaud (1993), Babadzhanov et al. (2004), King (2012)and Mata et al. (2010).

## Chickens

Chickens have been almost the exclusive focus of research into *Spirulina's* usefulness in poultry feed rations (Table A2.3). For instance, Ross and Dominy (1990) found chicken growth rates declined when *Spirulina* replaced dehulled soybean meal in rations, at either 10% or 20%. Paradoxically, studies which replaced groundnut cake (Saxena et al., 1983) or fishmeal (Venkataraman et al., 1994) with *Spirulina* in

chicken diets found no variation in typical growth. Therefore, from these studies, we can form a conclusion that dietary *Spirulina's* influence on chicken growth and growth rates depends on the feed-type it replaces in a ration. Although, it has been shown that dietary *Spirulina* levels of 50-100 g/kg of feed ration will maintain typical growth rates, yet levels exceeding 200 g/kg will bring about declined growth rates (Toyomizu et al., 2001).

**Table A2.3** Studies on the effects of *Spirulina* on growth and health of chickens

| Parameter | Summary of results | References(s) |
|---|---|---|
| *Growth* | Growth rates declined in 3 week old chicks fed *Spirulina* levels of 10% and 20% of diet | (Ross and, Dominy, 1990) |
| | Body weights of chicks fed *Spirulina* levels of 11.1 and 16.6% of diet were not different from the control group, receiving groundnut cake | (Saxena et al., 1983) |
| | Broilers fed *Spirulina* levels of 140 and 170 g/kg of diet and vitamin and mineral premixes omitted had no difference in dressing percentage compared to those receiving fishmeal or groundnut cake | (Venkataraman et al., 1994) |
| | Broilers fed *Spirulina* levels of 0, 40, or 80 g/kg of diet for 16 days did not significantly differ in body weights | (Toyomizu et al., 2001) |
| | Broilers fed *Spirulina* levels of 40 g/kg of diet had greater muscle redness and yellowness than the control group | (Toyomizu et al., 2001) |
| | White Leghorn and broilers fed *Spirulina* levels of 0, 0.001, 0.1, 1 and 10 g/kg of diet had comparable body weights after 7 weeks | (Qureshi et al., 1996) |
| *Health* | Chicks fed *Spirulina* levels of 10 g/kg of diet had increased NK-cell activity compared to the control group, showing an enhanced disease resistance potential | (Qureshi et al., 1996) |
| | Chicken phagocytic activity had an incremental linear increase with increasing dietary *Spirulina* levels of 0.5, 1 and 2% of diet | (Al-Batshan et al., 2001) |
| *Product quality* | White Leghorn hens egg total cholesterol levels were reduced when diets contained 150 g flaxseeds + 200 mg vitamin E + 3 g *Spirulina* per kg diet | (Sujatha and, Narahari, 2011) |
| | White Leghorn layers, aged 32 weeks, fed 20% whole flaxseeds and 5% *Spirulina* (w/w) produced eggs with higher levels of linoleic acid with less cholesterol | (Rajesha et al., 2011) |
| | Egg yolk colour score was higher in layers fed flaxseed diets with 5% *Spirulina* (w/w) compared to those on a flaxseed diet (20% w/w) | (Rajesha et al., 2011) |
| | Optimal egg yolk pigmentation was obtained by feeding *Spirulina* levels of 1% of diet, when diet is otherwise free of xanthophylls | (Anderson et al., 1991) |
| | Egg yolk carotenoids pigment and omega-3 fatty acid levels increase when White Leghorn hens fed 150 g flaxseeds + 200 mg vitamin E + 3 g *Spirulina* per kg diet | (Sujatha and, Narahari, 2011) |

Dietary *Spirulina* has been associated with greater cost efficiency in chicken production. Venkataraman *et al.* (1994) found that vitamin-mineral premixes

normally added to chicken feed rations can be omitted when *Spirulina* is included, due to its nutrient-rich composition. Furthermore, chickens receiving dietary *Spirulina* have been found to be of better health than those not receiving *Spirulina* (Venkataraman et al., 1994). This finding is based upon increased macrophage and overall mononuclear phagocyte system functionality, both indicative of enhanced disease resistance, present with increased dietary *Spirulina* levels in chickens (Al-Batshan et al., 2001; Qureshi et al., 1996). Qureshi et al. (1996) found that improved chicken health can be inferred with just low dietary *Spirulina* levels, to 10 g/kg feed ration. Again, this promotes greater production cost efficiency.

*Spirulina* has been shown to be an effective means to alter chicken product qualities to meet consumer preferences. For instance, total cholesterol content of eggs can be lowered by including *Spirulina* into layer hen's feed rations (Sujatha and, Narahari, 2011). This arises from *Spirulina's* high antioxidant and omega-3 polyunsaturated fatty acids (PUFA) content, which enriches an egg's nutritional value at the expense of its cholesterol content (Rajesha et al., 2011; Sujatha and, Narahari, 2011). Egg yolk colour has also been found to intensify linearly with increased dietary *Spirulina* levels (Ross and, Dominy, 1990; Sujatha and, Narahari, 2011). In white Leghorn layer hens, dietary *Spirulina* levels of 3-9% total feed ration was found to result in egg yolk colours best representative of consumer preferences (Saxena et al., 1983). Similar findings have been found in trials with Japanese quails (Ross *et al.*, 1994). Spirulina's effect on yolk colour results from its content of high levels of zeaxanthin, xanthophylls and other carotenoid pigments, particularly β-carotene, which accumulate within the yolk (Anderson et al., 1991; Takashi, 2003). These same compounds have been found to also accumulate within chicken's muscle tissue. Both Toyomizu et al. (2001) and Venkataraman et al. (1994) have reported this outcome with muscle tissue increasing in yellowness and redness with increasing levels of dietary *Spirulina*. Dietary *Spirulina* levels at 1% total diet rations in the week prior to slaughter has been found to result in broiler muscle tissue pigmentation at levels best representing consumer preferences (Dismukes et al., 2008).

**Pigs**

Research into pig growth responses to dietary *Spirulina* current proves inconclusive (Table A2.4). Hugh et al. (1985) found that crossbred weanling pigs receiving dietary *Spirulina* had growth rates of up to 9% higher than those receiving no *Spirulina*. However, Grinstead et al. (1998), found no growth difference in his experimental pigs receiving and receiving no *Spirulina*. Yet, this divergence in findings is thought to result from differences in experimental procedures.

**Table A2.4** Studies on the effects of *Spirulina* on growth and health of pigs

| Parameter | Summary of results | Reference(s) |
|---|---|---|
| *Growth* | Crossbred weanling pigs fed *Spirulina* levels of 1.5 and 3% of diet had higher growth rates to the control group | (Hugh et al., 1985) |
| | Weanling pigs fed *Spirulina* pelleted diets had decreased average daily gain (ADG) while those receiving Spirulina in meal diets had improved ADG | (Grinstead *et al.*, 2000)<br>(Grinstead et al., 1998) |
| | ADG in pigs fed *Spirulina* levels of 2% of diet was greater than the control group, during days 14-28 post-weaning | (Grinstead et al., 2000)<br>(Grinstead et al., 1998) |
| | Pigs fed *Spirulina* levels of 14% of diet had similar growth as those fed skim milk powder | (Grinstead et al., 1998) |
| | Increasing *Spirulina* levels in pig diets (0.5, 1 and 2% diet) showed only a numerical increase in ADG | (Grinstead et al., 1998) |
| *Fertility* | Boars fed BioR (extracted from *Spirulina*) at 1.5 mL/day had increased ejaculate volume and spermatozoa mobility compared to a control group | (Granaci, 2007a) |

Different pig types were used by Hugh et al. (1985) and Grinstead et al. (1998), with the heterosis introduced through crossbreeding potentially effecting the growth observed (Gillespie and, Flanders, 2010). Another explanation arises from findings that dietary protein digestibility will decrease within increasing levels of *Spirulina*, in pigs (Fevrier and, Seve, 1975). This is due to *Spirulina's* complex cell wall structure resisting pig's digestive enzymes (Fevrier and, Seve, 1975), themselves varying between pig breeds. Differences in basal pig diets also existed (Grinstead et al., 1998; Hugh et al., 1985). This would affect any growth response, as would the form in which the dietary *Spirulina* was provided. For instance, a difference in growth was shown between pigs fed pelletised and non-pelletised *Spirulina* (Grinstead et al., 2000; Grinstead et al., 1998). Pig health has also been suggested as a causal factor of the different outcomes in growth trials with *Spirulina* (Grinstead et al., 2000; Grinstead et al., 1998). Essentially, *Spirulina's* usefulness in pig feeds will depend on

the feed-type it is replacing. For instance, *Spirulina* was been demonstrated to be a viable replacement for dried skim milk powder in pig feed rations (Grinstead et al., 1998).

An advantage to pig rations containing *Spirulina* revolves around boar fertility. Granaci (2007a) found that boar's receiving a *Spirulina* extract have greater overall sperm quality to those receiving no *Spirulina*; in terms of increased volume (11%), motility, and post-storage viability (5%).

**Ruminants**

Ruminants' ability to digest unprocessed algal material (Gouveia et al., 2008) makes them especially suited to dietary *Spirulina*. This is further complemented by ruminants' efficient digestion of *Spirulina's* carbohydrate fraction, when used in levels up to 20% of total feed intake, compared to other algal feed-types, including *Chlorella* or *Scenedesmus obliquus* (Gouveia et al., 2008). *Spirulina* has been shown to increase microbial crude protein production and reduce its retention time within a rumen (Quigley and, Poppi, 2009). Furthermore, approximately 20% of dietary *Spirulina* bypasses rumen degradation, and is therefore available for direct absorption within the abomasums (Panjaitan et al., 2010; Quigley and, Poppi, 2009; Zhang *et al.*, 2010).

When *Spirulina* is delivered to ruminants as a water suspension, it has been found to be preferentially consumed when compared to pure water (Panjaitan et al., 2010). Moreover, *Spirulina's* high sodium content increases ruminant's water consumption and urine excretion (Panjaitan et al., 2010), although this is typical of algal feed-types (Marin *et al.*, 2009).

*Cattle*

*Spirulina* trials using dairy cows have shown results with positive implications on productivity (Table A2.5). Kulpys et al. (2009) found that cows receiving dietary

*Spirulina* had a 21% increase in their milk production. Furthermore, Simkus et al. (2007; 2008) showed an increase in milk fat (between 17.6% and 25.0%), milk protein (up by 9.7%) and lactose (up by 11.7%) in cows receiving *Spirulina* compared to those receiving no *Spirulina*. Milk's saturated fatty acids content decreased and mono- and poly-unsaturated fatty acids increased when cows received *Spirulina* (Christaki et al., 2012). These results may occur due to *Spirulina's* aforementioned influence on microbial protein synthesis, avoidance of rumen degradation, and its nutrient-rich composition. Moreover, these findings highlight *Spirulina's* use in enhancing milk's health appeal.

Dietary *Spirulina* has also been associated with significant decreases in milk somatic cell count (Simkus et al., 2007), hence improving milk's value. Additionally, dairy cows receiving *Spirulina* have been found to have improved body condition (8.5-11%) when compared to others receiving no *Spirulina* (Kulpys et al., 2009).

As found with pigs, bull sperm quality has been shown to be improved with *Spirulina*. Sperm motility, concentration and post-storage viability were all positively affected when bulls received a bio-extract removed from *Spirulina* (Granaci, 2007b). However, the effect of 'raw' dietary *Spirulina* on bull sperm quality needs to be further studied.

*Sheep*

Research into sheep production responses to dietary *Spirulina* is in its infancy (Table A2.5). Nonetheless, Bezerra et al. (2010) has found that lambs receiving *Spirulina* have higher liveweights and average daily weight gains (ADG) than other receiving no *Spirulina*. Findings from Holman et al. (2012) also show an increase in lamb liveweight with dietary *Spirulina*, along with an increase in body condition and many other body conformation traits. However, ADG findings did not reflect each other. This divergence is thought to results from differences in lamb ages and *Spirulina* suspensions used to deliver the *Spirulina*, between these trials.

Shimkiene et al. (2010) has shown that pregnant ewes receiving *Spirulina* will deliver heavier lambs (up 4.07%) with greater ADG compared to pregnant ewes receiving no *Spirulina*. However, delivering *Spirulina* to pregnant ewes is problematic.

**Table A2.5** Studies on the effects of *Spirulina* on growth and health of ruminants

| Species | Parameter | Summary of results | Reference(s) |
|---|---|---|---|
| Cow | *Growth* | Dairy cows fed 200 g *Spirulina* daily were 8.5-11% fatter than the control group, evaluated using body condition score | (Kulpys et al., 2009) |
| | *Productivity* | Dairy cows fed 200 g *Spirulina* daily produced more milk than the control group | (Kulpys et al., 2009) |
| | | Cows fed *Spirulina* levels of 2g/day (w/w) produced more milk than the control group | (Simkus et al., 2007) |
| | | *Spirulina* levels of 0.15% of diet resulted in decreased rumen degradability of dietary crude protein | (Zhang et al., 2010) |
| | *Product Quality* | Milk from cows fed *Spirulina* levels of 2g/day had greater average milk fat, protein, and lactose than controls | (Simkus et al., 2007)<br>(Simkus et al., 2008) |
| | | Milk saturated fatty acid levels decreased while mono- and polyunsaturated fatty acids increased when crossbred Holsteins were fed *Spirulina* at 40 g/day | (Christaki et al., 2012) |
| | | *Spirulina* fed at 2g/day to dairy cows reduces the somatic cell counts | (Simkus et al., 2007) |
| Sheep | *Growth* | 6 month old lambs fed *Spirulina* levels of 10% (w/w) had greater liveweights than those given 20% (w/w) and the control group | (Holman et al., 2012) |
| | | Lambs body condition scores incrementally higher in lambs fed *Spirulina* levels of 10 and 20% (w/w) compared to controls | (Holman et al., 2012) |
| | | Lambs fed cow milk enriched with 10 g/day *Spirulina* had higher liveweights and growth rates during 15-30 days old than the control group | (Bezerra et al., 2010) |
| | | Pregnant ewes fed pellets containing 2g *Spirulina ad libitum* produced newborn lambs with higher weights and average daily gains than those from control treatment ewes | (Shimkiene et al., 2010) |

**Rabbits**

*Spirulina* has been trialled in the feed rations of commercially farmed meat rabbits (Table 6). Presently, its inclusion in rabbit diets has been shown to not influence rabbit growth (Peiretti and, Meineri, 2008) or carcass yields (Peiretti and, Meineri, 2011). These findings may quell concerns that feed rations containing *Spirulina* would be less digestible than conventional rabbit diets. However, rabbits receiving dietary *Spirulina* have an increased total feed consumption compared to those receiving no *Spirulina* (Peiretti and, Meineri, 2008). This suggests that dietary *Spirulina's* non-effect on rabbit growth or carcass yields may result from an overall increase in feed intake. Although, dietary *Spirulina* levels of 1% total feed intake was

found to improve crude protein digestibility in rabbits fed both low and high fat diets, comparative to those receiving no *Spirulina* (Peiretti and, Meineri, 2009). Hence, including *Spirulina* into rabbit diets may prove useful when basal diets are high in fat; to provide sufficient energy to 'fuel' optimal growth rates (Peiretti and, Meineri, 2009).

**Table A2.6** Studies on the effects of *Spirulina* on growth and health of rabbits

| Parameter | Summary of results | Reference(s) |
|---|---|---|
| *Growth* | Final weight and weight gain did not differ between rabbits fed *Spirulina* levels of 0, 5, 10, or 15% of diet | (Peiretti and, Meineri, 2008)<br>(Peiretti and, Meineri, 2011) |
| | Feed intake of rabbits fed *Spirulina* levels of 5 and 10% of diet was greater than the control and 15% groups | (Peiretti and, Meineri, 2011) |
| | Rabbits receiving *Spirulina* levels of 1% of diet had increased crude protein digestibility in both low and high fat diets | (Peiretti and, Meineri, 2009) |
| | *Spirulina* levels of 10% of diet resulted in high feed intake compared to control group | (Peiretti and, Meineri, 2008) |
| *Health* | New Zealand White rabbits fed a high fat diet and supplemented *Spirulina* levels of 10 g/kg of diet had reduced reactive oxygen species and oxidative stress | (Meineri et al., 2009) |
| *Product Quality* | Gamma-linoleic acid content in the peri renal fat and meat tissue in rabbits increased with *Spirulina* levels of 5, 10 and 15% of diet | (Peiretti and, Meineri, 2011) |

Rabbit meat quality has been shown to improve when rabbits receive dietary *Spirulina*. For instance, Meineri et al. (2009) and Peirette and Meineri (Peiretti and, Meineri, 2011) both identified dietary *Spirulina* as a causal factor increasing gamma-linolenic acid (GLA) and n-6/n-3 PUFA ratios within rabbit muscle lipid contents. This supports continued consumer preferable meat colour and appearance by improving rabbit meat's oxidative stability (Dalle Zotte and, Szendro, 2011). Furthermore, GLA has health benefits for humans (Howe et al., 2006), and its increased level in rabbit meat would appeal to health conscious consumers. Rabbit health has also been found to improve with dietary *Spirulina*, as rabbits receiving *Spirulina* had greater blood oxygen levels than those receiving no *Spirulina* (Meineri et al., 2009).

**Conclusion**

*Spirulina* is a promising new feed source to support future animal production needs. Trials using dietary *Spirulina* in feed rations of many agriculturally significant animal

species have already shown improvements in productivity, health, and product quality. However, many results contradict other findings, and together present a convoluted picture of *Spirulina's* usefulness as an animal feed. Consequently, further research with *Spirulina* as a feed source, in discussed animal species and other animal species; such as beef cattle, goats, llama, alpaca and deer, is needed to clarify its potential. Furthermore, investigations into *Spirulina's* active ingredients and associated biological pathways would aid in broadening our knowledge, scope and applicable ramifications of *Spirulina* usage in sustainable animal production into the foreseeable future.

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*Appendix 3*

# Additional Articles

The following tables are **supplementary results** which present data which was deemed unnecessary to inclusion into corresponding Thesis chapters as they fail to contribute further information availing to the acceptance or rejection of predetermined hypotheses.

## Chapter 2: Physical wool traits with high and low planes of nutrition

### **S2.1** *Spirulina* supplementation level and sex interactions on change in physical wool traits [1]

| | *Units* | CONTROL | | | | LOW | | | | MEDIUM | | | | HIGH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔFD | c | -0.93 | 1.75 | -0.47 | 1.01 | 1.54 | 0.37 | 0.60 | 1.08 | 2.06 | 1.56 | -1.27 | 0.50 | 0.49 | 0.82 | 1.23 | 0.50 |
| ΔCV | % | 3.90 | 2.44 | 2.17 | 1.19 | -0.20 | 0.38 | 0.13 | 1.09 | -1.41 | 0.59 | -0.01 | 0.82 | 1.50 | 1.68 | -0.37 | 0.82 |
| ΔSD | . | 0.83 | 0.39 | 0.47 | 0.30 | 0.23 | 0.04 | 0.14 | 0.39 | 0.11 | 0.33 | -0.26 | 0.18 | 0.41 | 0.28 | 0.11 | 0.14 |
| ΔCF | % | 1.20 | 9.07 | 1.13 | 4.96 | -6.16 | 1.31 | -4.50 | 3.50 | -5.71 | 5.90 | 0.11 | 0.10 | -3.57 | 2.63 | -7.47 | 2.20 |
| ΔSF | µm | -0.03 | 1.23 | 0.00 | 0.93 | 1.43 | 0.28 | 2.29 | 0.68 | 1.77 | 1.51 | -1.21 | 0.50 | 0.76 | 0.49 | 1.10 | 0.34 |
| ΔCURV | °/mm | -2.33 | 0.88 | -7.67 | 7.69 | -4.43 | 1.99 | -7.00 | 4.19 | 1.00 | 2.28 | 2.14 | 3.81 | -7.14 | 2.14 | -3.43 | 4.90 |
| ΔYIELD | % | -1.27 | 0.99 | -4.87 | 3.50 | 0.41 | 1.70 | -2.37 | 2.02 | 0.07 | 2.12 | -1.73 | 2.29 | 0.79 | 1.05 | 0.26 | 2.60 |

[1] Change in (Δ): mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), clean fleece percentage (YIELD).

### **S2.2** *Spirulina* supplementation level and sire breed interactions on change in physical wool traits [1]

| | ΔFD (µm) | | ΔCV (%) | | ΔSD | | ΔCF (%) | | ΔSF (µm) | | ΔCURV (°/mm) | | ΔYIELD (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CONTROL** | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Black Suffolk | -0.05 | 1.35 | 2.50 | 0.20 | 0.60 | 0.30 | -1.75 | 6.55 | 0.45 | 1.35 | -12.00 | 11.00 | -6.95 | 4.55 |
| Dorset | 0.17 | 1.93 | -1.20 | 1.32 | -0.23 | 0.56 | -3.03 | 4.43 | 2.88 | 1.03 | 1.50 | 3.30 | -3.50 | 2.69 |
| Merino | 1.80 | 3.23 | -0.08 | 1.06 | 0.30 | 0.58 | -10.13 | 10.33 | 1.70 | 3.04 | 0.50 | 4.73 | -1.48 | 2.34 |
| White Suffolk | -0.53 | 1.25 | 3.83 | 2.61 | 0.80 | 0.39 | -2.60 | 3.58 | 0.23 | 0.65 | -8.50 | 3.01 | -1.48 | 2.81 |
| **LOW** | | | | | | | | | | | | | | |
| Black Suffolk | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Dorset | 1.15 | 0.75 | 1.00 | 0.20 | 0.45 | 0.15 | -4.90 | 1.60 | 1.25 | 0.65 | -9.00 | 2.00 | 1.50 | 1.00 |
| Merino | 0.90 | 0.80 | -1.55 | 0.65 | -0.15 | 0.25 | 0.25 | 0.15 | 0.70 | 0.80 | -1.00 | 2.00 | 3.95 | 3.55 |
| White Suffolk | 2.65 | 0.55 | -2.35 | 1.05 | -0.10 | 0.20 | -6.90 | 6.60 | 2.10 | 0.30 | 4.50 | 7.50 | 1.60 | 2.30 |
| **MEDIUM** | | | | | | | | | | | | | | |
| Black Suffolk | 0.05 | 2.25 | 2.30 | 1.60 | 0.60 | 0.00 | -3.75 | 12.25 | 0.55 | 1.85 | -0.50 | 1.50 | -2.80 | 0.70 |
| Dorset | 1.40 | 0.74 | 1.35 | 0.92 | 0.58 | 0.25 | -8.83 | 4.66 | 1.60 | 0.69 | -10.25 | 3.38 | -0.98 | 2.03 |
| Merino | -0.07 | 0.50 | 0.22 | 1.25 | 0.00 | 0.20 | 0.03 | 0.17 | -0.08 | 0.42 | 4.50 | 2.53 | -4.78 | 3.18 |
| White Suffolk | 0.80 | 0.62 | -0.80 | 0.68 | -0.08 | 0.15 | -4.33 | 3.58 | 0.65 | 0.52 | -1.75 | 5.68 | 1.87 | 3.75 |
| **HIGH** | | | | | | | | | | | | | | |
| Black Suffolk | -2.10 | 1.60 | 4.30 | 4.40 | 0.75 | 0.85 | 9.00 | 5.80 | -1.05 | 0.55 | -2.50 | 1.50 | 0.55 | 0.15 |
| Dorset | 1.60 | 0.27 | -0.78 | 0.82 | 0.08 | 0.16 | -4.35 | 1.29 | 1.40 | 0.15 | -6.75 | 5.27 | 0.30 | 3.39 |
| Merino | -0.80 | 0.75 | -1.88 | 0.62 | -0.48 | 0.21 | 0.18 | 0.19 | -1.00 | 0.77 | 1.00 | 5.96 | 1.38 | 2.56 |
| White Suffolk | 1.40 | 0.36 | 0.13 | 0.35 | 0.25 | 0.12 | -8.95 | 2.25 | 1.33 | 0.34 | -10.50 | 4.56 | 0.63 | 1.63 |

[1] Change in (Δ): mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), clean fleece percentage (YIELD).

### **S2.3** Sire breed and sex interactions on change in physical wool traits [1]

| | *Units* | Black Suffolk | | | | Dorset | | | | Merino | | | | White Suffolk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔFD | c | 1.90 | 1.80 | -1.09 | 0.91 | 1.40 | 0.51 | 1.73 | 0.90 | 0.77 | 0.33 | 0.46 | 0.76 | 0.41 | 0.76 | 0.24 | 0.65 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ΔCV | % | 1.37 | 1.77 | 0.80 | 1.02 | -0.47 | 0.07 | -1.47 | 1.38 | -0.47 | 0.57 | 1.57 | 0.81 | 0.86 | 1.40 | -1.07 | 0.52 |
| ΔSD | . | 0.67 | 0.35 | 0.00 | 0.36 | 0.17 | 0.07 | -0.03 | 0.32 | 0.04 | 0.14 | 0.41 | 0.19 | 0.33 | 0.25 | 0.20 | 0.16 |
| ΔCF | % | -8.44 | 6.04 | -1.06 | 2.16 | -2.23 | 4.54 | -5.47 | 6.46 | -3.37 | 2.32 | -5.20 | 3.89 | -2.16 | 3.43 | -2.77 | 1.73 |
| ΔSF | µm | 2.10 | 1.54 | 0.77 | 1.01 | 1.30 | 0.36 | 1.40 | 0.76 | 0.67 | 0.35 | 0.73 | 0.66 | 0.61 | 0.51 | 0.07 | 0.63 |
| ΔCURV | °/m | -5.71 | 2.09 | -1.43 | 4.66 | -4.33 | 1.33 | 0.67 | 6.64 | -3.43 | 2.97 | -1.00 | 3.96 | -0.57 | 1.99 | -9.43 | 4.53 |
| ΔYIELD | % | 0.04 | 1.12 | -5.71 | 2.02 | 3.10 | 2.39 | 1.60 | 1.15 | -1.13 | 1.50 | -1.89 | 2.83 | 0.49 | 0.91 | 0.99 | 1.52 |

[1] Change in (Δ): mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), clean fleece percentage (YIELD)

## **S2.4** *Spirulina* supplementation level and plane of nutrition interactions on change in physical wool traits [1]

| | | High | | | | | | Low | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Units* | CONTROL | | MEDIUM | | HIGH | | CONTROL | | LOW | | MEDIUM | | HIGH | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔFD | c | 0.26 | 1.86 | 1.10 | 0.63 | 0.44 | 0.71 | 0.60 | 0.58 | 1.57 | 0.47 | -0.03 | 0.31 | 0.18 | 0.69 |
| ΔCV | % | 1.61 | 1.67 | 0.21 | 0.90 | 0.45 | 1.24 | 0.38 | 0.72 | -0.97 | 0.72 | 1.00 | 0.53 | -0.85 | 0.68 |
| ΔSD | . | 0.45 | 0.45 | 0.24 | 0.20 | 0.21 | 0.22 | 0.18 | 0.14 | 0.07 | 0.15 | 0.22 | 0.15 | -0.13 | 0.23 |
| ΔCF | % | -5.19 | 5.72 | -6.20 | 3.75 | -2.04 | 3.19 | -4.17 | 1.68 | -3.85 | 2.21 | -1.73 | 0.74 | -3.03 | 1.25 |
| ΔSF | µm | 2.05 | 1.54 | 1.10 | 0.56 | 0.56 | 0.47 | 0.62 | 0.53 | 1.35 | 0.38 | 0.17 | 0.31 | 0.05 | 0.72 |
| ΔCURV | °/mm | -6.63 | 3.31 | -0.63 | 2.60 | -4.63 | 2.34 | 0.50 | 3.52 | -1.83 | 3.23 | -4.33 | 4.56 | -5.50 | 5.75 |
| ΔYIELD | % | -5.84 | 1.60 | -5.04 | 1.36 | -2.30 | 0.98 | 1.17 | 0.95 | 2.35 | 1.23 | 3.20 | 1.79 | 4.78 | 0.91 |

[1] Change in (Δ): mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), clean fleece percentage (YIELD)

## **S2.5** Sire breed and sex interactions with plane of nutrition on change in physical wool traits [1]

| | | High | | | | Low | | | | High | | | | | | Low | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Units* | Ewe | | Wether | | Ewe | | Wether | | Black Suffolk | | Dorset | | Merino | | White Suffolk | | Dorset | | Merino | | White Suffolk | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔFD | c | 1.23 | 1.13 | -0.03 | 0.72 | 0.93 | 0.24 | 0.23 | 0.50 | -0.70 | 0.91 | 1.13 | 1.35 | 1.68 | 1.98 | 0.28 | 1.00 | 1.03 | 0.24 | -0.58 | 0.54 | 1.29 | 0.36 |
| ΔCV | % | 1.20 | 1.26 | 0.32 | 0.79 | -0.29 | 0.40 | 0.08 | 0.60 | 3.03 | 1.28 | -1.47 | 0.93 | -0.42 | 0.80 | 1.88 | 2.05 | 1.04 | 0.40 | -0.94 | 0.73 | -0.43 | 0.50 |
| ΔSD | . | 0.53 | 0.25 | 0.08 | 0.24 | 0.13 | 0.09 | 0.04 | 0.14 | 0.65 | 0.23 | -0.13 | 0.41 | 0.22 | 0.39 | 0.47 | 0.34 | 0.43 | 0.08 | -0.29 | 0.14 | 0.11 | 0.08 |
| ΔCF | % | -5.98 | 4.13 | -2.98 | 2.69 | -2.73 | 1.01 | -3.66 | 1.16 | 1.17 | 4.62 | -7.02 | 4.26 | -6.78 | 6.86 | -5.27 | 3.64 | -4.06 | 0.60 | -0.19 | 1.00 | -5.71 | 1.62 |
| ΔSF | µm | 1.45 | 0.94 | 1.03 | 0.64 | 0.85 | 0.24 | 0.24 | 0.46 | -0.02 | 0.69 | 2.78 | 0.70 | 1.53 | 1.88 | 0.65 | 0.60 | 1.16 | 0.18 | -0.66 | 0.50 | 1.14 | 0.27 |
| ΔCURV | °/m | -3.75 | 1.79 | -4.17 | 2.79 | -3.00 | 1.74 | -2.58 | 3.92 | -5.00 | 3.66 | -4.67 | 2.53 | -2.00 | 3.18 | -4.17 | 4.16 | -6.50 | 3.59 | -4.25 | 2.66 | -6.13 | 3.58 |
| ΔYIELD | % | -2.63 | 0.68 | -6.16 | 1.29 | -3.05 | 0.94 | -2.70 | 0.94 | -3.07 | 1.82 | -5.57 | 1.26 | -5.82 | 1.54 | -3.12 | 1.82 | -2.46 | 0.96 | -2.91 | 1.25 | -3.25 | 1.29 |

[1] Change in (Δ): mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), clean fleece percentage (YIELD)

## **S2.6** *Spirulina* supplementation level and sire breed interactions with sex on change in physical wool traits [1]

| | Ewe | | | | | | | | | | | | | | Wether | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ΔFD | | ΔCV | | ΔSD | | ΔCF | | ΔSF | | ΔCURV | | ΔYIELD | | ΔFD | | ΔCV | | ΔSD | | ΔCF | | ΔSF | | ΔCURV | | ΔYIELD | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |

| | | |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| e | 7 | 7 | 5 | 4 | 4 | 0 | 0 | 2 | 6 | 5 | . | 5 | . | 8 | 1 | 3 | . | 5 | 1 | 2 | . | 9 | 0 | 4 | 5 | 5 | 4 | 2 |
| Su | 0 | 0 | 0 | 0 | 0 | 0 | . | 0 | 5 | 5 | 5 | 0 | 1 | 5 | 0 | 0 | 2 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | . | 0 | 0 | 0 |
| ffo | | | | | | | 4 | | | | 0 | | 5 | | | | 5 | | | | 0 | | | | 5 | | | |
| lk | | | | | | | 0 | | | | | | | | | | | | | | | | | | 0 | | | |

[1] Change in (Δ): mean fibre diameter (FD), fibre diameter coefficient of variation (CV), fibre diameter standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), clean fleece percentage (YIELD)

## Chapter 3: Physical wool traits with high plane of nutrition

### S3.1 *Spirulina* supplementation level and sire breed interactions on physical wool traits under high plane of nutrition [1]

| | Units | CONTROL | | | | | | | | MEDIUM | | | | | | | | HIGH | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Black Suffolk | | Dorset | | Merino | | White Suffolk | | Black Suffolk | | Dorset | | Merino | | White Suffolk | | Black Suffolk | | Dorset | | Merino | | White Suffolk | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| FD | µm | 25.54 | 0.96 | 23.09 | 1.04 | 20.92 | 3.233 | 26.61 | 1.16 | 28.48 | 0.84 | 25.56 | 0.83 | 26.61 | 0.70 | 27.81 | 0.75 | 27.00 | 0.96 | 26.15 | 1.18 | 21.16 | 0.70 | 26.21 | 0.65 |
| CV | % | 19.80 | 2.49 | 20.43 | 0.43 | 18.53 | 0.39 | 20.78 | 0.38 | 18.87 | 0.87 | 15.84 | 0.54 | 18.23 | 0.41 | 19.98 | 0.38 | 20.00 | 2.58 | 18.53 | 0.36 | 16.50 | 0.58 | 19.55 | 0.59 |
| SD | . | 5.10 | 0.37 | 4.40 | 0.50 | 3.73 | 0.54 | 5.33 | 0.46 | 5.30 | 0.17 | 4.03 | 0.24 | 4.80 | 0.23 | 5.60 | 0.23 | 5.13 | 0.51 | 4.95 | 0.73 | 3.98 | 0.23 | 5.13 | 0.13 |
| CF | % | 83.94 | 4.63 | 90.40 | 1.83 | 91.54 | 1.73 | 89.72 | 2.02 | 93.09 | 1.97 | 94.03 | 1.46 | 94.85 | 1.24 | 92.73 | 1.03 | 87.55 | 4.75 | 90.79 | 1.79 | 91.54 | 1.73 | 89.72 | 2.02 |
| SF | µm | 24.35 | 1.12 | 21.38 | 1.64 | 19.32 | 3.033 | 24.57 | 1.45 | 26.34 | 0.97 | 23.70 | 1.77 | 24.57 | 0.90 | 25.75 | 0.96 | 25.00 | 1.90 | 23.54 | 1.43 | 19.32 | 0.90 | 24.20 | 0.84 |
| CURV | °/mm | 73.38 | 3.97 | 69.50 | 4.11 | 66.68 | 2.682 | 75.46 | 2.52 | 72.64 | 1.31 | 69.75 | 3.17 | 73.13 | 3.50 | 72.73 | 1.61 | 77.18 | 1.35 | 72.25 | 2.49 | 75.38 | 3.95 | 71.93 | 0.80 |
| YIELD | % | 74.48 | 1.25 | 75.46 | 2.52 | 77.32 | 2.092 | 73.38 | 3.23 | 72.64 | 1.31 | 72.73 | 1.61 | 74.48 | 1.25 | 75.46 | 2.52 | 77.18 | 1.35 | 71.93 | 0.80 | 77.32 | 2.09 | 73.38 | 3.23 |

[1] Mean fibre diameter (FD), coefficient of variation (CV), standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), and clean fleece yield (YIELD)

### S3.2 *Spirulina* supplementation level and sex on physical wool traits under high plane of nutrition [1]

| | Units | CONTROL | | | | MEDIUM | | | | HIGH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| FD | µm | 25.08 | 1.21 | 22.41 | 1.73 | 25.09 | 1.92 | 24.11 | 1.76 | 25.00 | 1.90 | 23.54 | 1.43 |
| CV | % | 20.59 | 1.36 | 18.48 | 0.61 | 17.44 | 0.66 | 18.18 | 0.80 | 18.53 | 1.36 | 16.65 | 0.71 |
| SD | . | 5.14 | 0.34 | 4.14 | 0.34 | 4.33 | 0.30 | 4.41 | 0.41 | 4.70 | 0.56 | 3.86 | 0.16 |
| CF | % | 81.51 | 4.21 | 89.43 | 3.94 | 80.09 | 7.50 | 83.54 | 4.58 | 77.96 | 5.73 | 91.38 | 2.82 |
| SF | µm | 24.35 | 1.12 | 21.38 | 1.64 | 23.70 | 1.77 | 22.98 | 1.72 | 23.95 | 1.91 | 22.09 | 1.27 |
| CURV | °/mm | 73.38 | 3.97 | 69.50 | 4.11 | 69.75 | 3.17 | 73.13 | 3.50 | 72.25 | 2.49 | 75.38 | 3.95 |
| YIELD | % | 74.48 | 1.25 | 75.46 | 2.52 | 72.64 | 1.31 | 72.73 | 1.61 | 77.18 | 1.35 | 71.93 | 0.80 |

[1] Mean fibre diameter (FD), coefficient of variation (CV), standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), and clean fleece yield (YIELD)

**S3.3** Sire breed and sex interactions on physical wool traits under high plane of nutrition [1]

| | *Units* | Black Suffolk | | | | Dorset | | | | Merino | | | | White Suffolk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| FD | µm | 28.27 | 1.13 | 26.10 | 0.45 | 26.45 | 0.93 | 23.48 | 1.35 | 19.32 | 2.10 | 16.70 | 0.38 | 26.18 | 0.74 | 27.13 | 0.46 |
| CV | % | 20.22 | 1.42 | 18.52 | 0.96 | 19.20 | 0.95 | 17.03 | 0.92 | 17.75 | 0.84 | 18.43 | 0.66 | 18.23 | 2.17 | 17.08 | 0.86 |
| SD | . | 5.65 | 0.23 | 4.83 | 0.25 | 5.10 | 0.38 | 4.00 | 0.32 | 3.43 | 0.41 | 3.08 | 0.15 | 4.70 | 0.40 | 4.63 | 0.24 |
| CF | % | 65.47 | 6.52 | 83.47 | 2.56 | 79.60 | 5.65 | 91.22 | 4.30 | 92.63 | 6.91 | 99.75 | 0.08 | 81.72 | 2.05 | 78.02 | 3.62 |
| SF | µm | 27.32 | 0.84 | 24.90 | 0.46 | 25.40 | 1.01 | 22.15 | 1.29 | 18.33 | 2.00 | 15.93 | 0.39 | 24.95 | 0.36 | 25.60 | 0.48 |
| CURV | °/mm | 67.83 | 2.01 | 80.83 | 4.23 | 79.17 | 4.24 | 71.17 | 2.90 | 65.17 | 1.85 | 61.83 | 2.24 | 75.00 | 3.37 | 76.83 | 3.99 |
| YIELD | % | 76.63 | 1.15 | 71.80 | 2.04 | 74.37 | 1.45 | 73.87 | 2.01 | 74.90 | 2.67 | 75.53 | 2.39 | 73.15 | 0.72 | 72.27 | 2.02 |

[1] Mean fibre diameter (FD), coefficient of variation (CV), standard deviation (SD), comfort factor (CF), spinning fineness (SF), fibre curvature (CURV), and clean fleece yield (YIELD)

## Chapter 4: Liveweight, body conformation and growth with high and low planes of nutrition

### **S4.1** Sire breed and sex on change in liveweight, body conformation and growth [1]

| | *Units* | Black Suffolk | | | | Dorset | | | | Merino | | | | White Suffolk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔCG | cm | 0.15 | 0.76 | 0.80 | 0.33 | 0.10 | 0.66 | 0.12 | 0.56 | -0.78 | 1.17 | -0.52 | 0.65 | -0.37 | 0.75 | 0.87 | 0.41 |
| ΔWH | cm | 0.00 | 0.39 | 0.15 | 0.64 | 0.04 | 0.39 | 0.18 | 0.30 | -0.68 | 0.64 | 0.08 | 0.47 | -0.71 | 0.61 | 0.35 | 0.27 |
| ΔBL | cm | 0.00 | 0.47 | 0.25 | 0.34 | -0.32 | 0.67 | 0.12 | 0.47 | -0.56 | 0.64 | 0.08 | 0.41 | -0.31 | 0.38 | 0.61 | 0.34 |
| ΔBCS | 1-5 | 0.05 | 0.14 | -0.05 | 0.12 | -0.04 | 0.10 | 0.00 | 0.09 | -0.02 | 0.07 | 0.10 | 0.06 | 0.00 | 0.11 | 0.07 | 0.07 |
| ΔBWT | kg | -0.25 | 1.02 | 1.35 | 0.42 | -0.22 | 0.73 | 0.88 | 0.48 | -1.18 | 0.76 | 0.44 | 0.44 | -0.50 | 0.71 | 0.7 | 0.49 |
| ADG | g/day | 121.77 | 31.98 | 140.14 | 48.48 | 76.09 | 35.71 | 93.00 | 34.78 | 81.63 | 23.49 | 97.38 | 30.72 | 87.17 | 29.52 | 84.26 | 36.57 |

[1] Change in (Δ): chest girth (CG), wither height (WH), body length (BL), body condition score (BCS), liveweight (BWT), average daily liveweight gain (ADG)

### **S4.2** Plane of nutrition interactions with sire breed and sex on change in liveweight, body conformation and growth [1]

| | *Units* | High | | | | Low | | | | High | | | | | | | | Low | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Black Suffolk | | Dorset | | Merino | | White Suffolk | | Dorset | | Merino | | White Suffolk | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔCG | cm | -0.84 | 0.75 | 0.29 | 0.38 | 0.29 | 0.49 | 0.17 | 0.43 | 0.48 | 0.41 | -0.25 | 0.60 | -1.24 | 1.38 | -0.17 | 0.79 | 0.39 | 0.61 | -0.21 | 0.56 | 0.50 | 0.52 |
| ΔWH | cm | -0.74 | 0.43 | 0.15 | 0.27 | -0.05 | 0.38 | 0.14 | 0.28 | 0.08 | 0.37 | -0.25 | 0.46 | -0.38 | 0.54 | -0.69 | 0.70 | 0.39 | 0.24 | -0.39 | 0.57 | 0.14 | 0.33 |
| ΔBL | cm | -0.50 | 0.41 | 0.41 | 0.19 | -0.19 | 0.42 | 0.12 | 0.37 | 0.13 | 0.29 | -0.27 | 0.51 | -0.29 | 0.67 | 0.19 | 0.36 | 0.04 | 0.61 | -0.21 | 0.46 | 0.07 | 0.37 |
| ΔBCS | 1-5 | -0.07 | 0.07 | 0.03 | 0.05 | 0.05 | 0.07 | 0.06 | 0.06 | 0.00 | 0.09 | -0.07 | 0.09 | 0.02 | 0.08 | -0.05 | 0.10 | 0.20 | 0.10 | 0.05 | 0.05 | 0.09 | 0.10 |
| ΔBWT | kg | -1.06 | 0.60 | 0.70 | 0.36 | -0.10 | 0.48 | 0.46 | 0.33 | 0.55 | 0.57 | -0.32 | 0.69 | -0.52 | 0.86 | -0.55 | 0.83 | 0.84 | 0.56 | -0.29 | 0.48 | -0.00 | 0.46 |
| ADG | g/day | 140.99 | 17.84 | 143.37 | 24.80 | 32.31 | 4.213 | 51.87 | 25.70 | 130.95 | 28.72 | 144.56 | 25.94 | 140.82 | 28.03 | 152.38 | 38.59 | 39.54 | 7.792 | 51.02 | 25.33 | 35.71 | 27.35 |

[1] Change in (Δ): chest girth (CG), wither height (WH), body length (BL), body condition score (BCS), liveweight (BWT), average daily liveweight gain (ADG)

### **S4.3** *Spirulina* supplementation level and sex interactions on change in liveweight, body conformation and growth [1]

| | *Units* | CONTROL | | | | LOW | | | | MEDIUM | | | | HIGH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔCG | c | -0.79 | 1.13 | 0.72 | 0.46 | 0.55 | 0.99 | -0.82 | 0.75 | -0.23 | 0.71 | 0.17 | 0.62 | -0.20 | 0.68 | 0.29 | 0.51 |
| ΔW | % | - | 0.6 | 0.54 | 0.3 | - | 0.6 | - | 0.8 | -0.19 | 0.5 | -0.04 | 0.3 | - | 0.3 | 0.23 | 0.3 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H | | 0.38 | 6 | | 0 | 0.18 | 3 | 0.45 | 8 | | 7 | | 3 | 0.72 | 8 | | 0 |
| ΔBL | . | -0.65 | 0.62 | 0.63 | 0.20 | -0.27 | 0.71 | -0.36 | 0.88 | -0.17 | 0.46 | 0.29 | 0.28 | -0.24 | 0.59 | 0.17 | 0.51 |
| ΔBCS | % | -0.08 | 0.09 | 0.09 | 0.08 | -0.09 | 0.15 | 0.05 | 0.14 | 0.04 | 0.10 | 0.04 | 0.06 | 0.04 | 0.10 | 0.00 | 0.07 |
| ΔBWT | µm | -1.06 | 0.84 | 1.30 | 0.34 | 0.27 | 1.24 | -0.32 | 0.68 | -0.25 | 0.63 | 0.23 | 0.55 | -0.80 | 0.62 | 0.65 | 0.42 |
| ADG | °/m | 90.67 | 32.23 | 102.62 | 26.60 | 40.82 | 41.11 | 40.82 | 37.07 | 115.16 | 19.42 | 115.45 | 41.52 | 73.76 | 33.73 | 99.13 | 34.91 |

[1] Change in (Δ): chest girth (CG), wither height (WH), body length (BL), body condition score (BCS), liveweight (BWT), average daily liveweight gain (ADG)

## **S4.4** *Spirulina* supplementation level and sire breed interactions on change in liveweight, body conformation and growth [1]

| | ΔCG (cm) | | ΔWH (cm) | | ΔBL (cm) | | ΔBCS (1-5) | | ΔBWT (kg) | | ΔADG (g/day) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CONTROL** | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Black Suffolk | 0.71 | 0.42 | 0.07 | 0.93 | -0.07 | 0.38 | -0.07 | 0.17 | 0.79 | 0.51 | 172.45 | 50.06 |
| Dorset | 0.93 | 0.88 | 0.57 | 0.49 | 0.21 | 0.79 | 0.00 | 0.14 | 0.75 | 1.01 | 101.53 | 42.84 |
| Merino | -2.04 | 1.88 | -0.57 | 0.99 | -0.43 | 0.87 | -0.07 | 0.10 | -1.00 | 1.14 | 86.22 | 29.86 |
| White Suffolk | 0.46 | 0.73 | 0.14 | 0.64 | 0.07 | 0.37 | 0.11 | 0.12 | 0.04 | 0.82 | 64.29 | 44.24 |
| **LOW** | | | | | | | | | | | | |
| Dorset | 0.71 | 0.78 | 0.29 | 0.29 | 0.29 | 0.47 | 0.21 | 0.15 | 1.79 | 1.33 | 61.22 | 61.68 |
| Merino | 0.14 | 0.96 | 0.00 | 1.20 | -0.29 | 1.30 | 0.00 | 0.00 | 0.14 | 0.32 | 40.82 | 39.42 |
| White Suffolk | -1.13 | 1.34 | -1.13 | 1.01 | -0.88 | 0.99 | -0.25 | 0.23 | -1.75 | 1.31 | 20.41 | 40.62 |
| **MEDIUM** | | | | | | | | | | | | |
| Black Suffolk | 0.50 | 0.22 | 0.50 | 0.34 | 0.50 | 0.22 | 0.08 | 0.08 | 1.67 | 0.36 | 143.88 | 54.21 |
| Dorset | 0.81 | 0.46 | 0.23 | 0.48 | 0.46 | 0.29 | 0.04 | 0.09 | 0.73 | 0.41 | 115.82 | 28.72 |
| Merino | -1.30 | 1.11 | -0.53 | 0.65 | -0.67 | 0.69 | 0.03 | 0.09 | -0.93 | 0.95 | 93.88 | 41.85 |
| White Suffolk | 0.32 | 0.94 | -0.25 | 0.77 | 0.29 | 0.45 | 0.04 | 0.15 | -0.44 | 0.84 | 121.94 | 56.73 |
| **HIGH** | | | | | | | | | | | | |
| Black Suffolk | 0.21 | 1.13 | -0.29 | 0.47 | 0.00 | 0.72 | 0.00 | 0.19 | -0.64 | 1.46 | 76.53 | 44.57 |
| Dorset | -1.44 | 0.91 | -0.47 | 0.47 | -1.00 | 1.01 | -0.19 | 0.14 | -1.00 | 0.76 | 47.96 | 63.70 |
| Merino | 1.15 | 0.37 | -0.23 | 0.50 | 0.46 | 0.43 | 0.19 | 0.07 | 0.62 | 0.42 | 112.76 | 39.73 |
| White Suffolk | 0.65 | 0.77 | 0.00 | 0.54 | 0.62 | 0.46 | 0.12 | 0.06 | 0.62 | 0.68 | 103.57 | 33.82 |

[1] Change in (Δ): chest girth (CG), wither height (WH), body length (BL), body condition score (BCS), liveweight (BWT), average daily liveweight gain (ADG)

## **S4.5** *Spirulina* supplementation level and plane of nutrition interactions on change in liveweight, body conformation and growth [1]

| | | CONTROL | | | | LOW | | MEDIUM | | | | HIGH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Units* | Low- | | High- | | Low- | | Low- | | High- | | Low- | | High- | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| ΔCG | c | 1.00 | 0.65 | -0.89 | 0.99 | -0.14 | 0.62 | 0.25 | 0.62 | -0.23 | 0.68 | -0.19 | 0.70 | 0.21 | 0.53 |
| ΔWH | % | 0.38 | 0.65 | -0.20 | 0.46 | -0.32 | 0.53 | 0.25 | 0.10 | -0.38 | 0.56 | -0.10 | 0.40 | -0.38 | 0.33 |
| ΔBL | . | 0.38 | 0.38 | -0.38 | 0.54 | -0.32 | 0.55 | 0.10 | 0.43 | 0.04 | 0.35 | -029 | 0.80 | 0.14 | 0.33 |
| ΔBCS | % | 0.10 | 0.08 | -0.07 | 0.09 | -0.02 | 0.10 | 0.15 | 0.09 | -0.04 | 0.07 | 0.00 | 0.11 | 0.04 | 0.06 |
| ΔBWT | µm | 0.33 | 0.56 | -0.16 | 0.77 | -0.02 | 0.69 | 0.47 | 0.54 | -0.36 | 0.60 | -0.02 | 0.54 | -0.14 | 0.55 |
| ADG | °/m | 17.01 | 33.09 | 156.38 | 23.89 | 40.82 | 27.34 | 76.53 | 32.59 | 144.39 | 31.22 | 34.01 | 45.95 | 125.77 | 23.64 |

[1] Change in (Δ): chest girth (CG), wither height (WH), body length (BL), body condition score (BCS), liveweight (BWT), average daily liveweight gain (ADG)

## **S4.6** *Spirulina* supplementation level and sire breed interactions with sex on change in liveweight, body conformation and growth [1]

| | Ewe | Wether |
|---|---|---|

| | ΔCG | | ΔWH | | ΔBL | | ΔBCS | | ΔBWT | | ADG | | ΔCG | | ΔWH | | ΔBL | | ΔBCS | | ΔBWT | | ADG | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| **CONTROL** | | | | | | | | | | | | | | | | | | | | | | | | |
| Black | 0.6 | 0.8 | 0.6 | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.0 | 15.5 | 1.27 | 0.7 | 0.5 | -0.3 | 0.6 | -0.1 | 0.3 | -0.1 | 0.2 | 0.8 | 0.2 | 18.9 | 1.01 |
| Suffolk | 0.7 | 0.3 | 0.7 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.3 | 10.2 | 2.56 | 0.5 | 0.7 | 0.3 | 0.6 | 0.1 | 0.7 | 0.1 | 0.3 | 0.5 | 0.2 | 8.07 | 2.97 |
| Dorset | 0.2 | 0.1 | 0.4 | 0.0 | -0.1 | 0.1 | -0.0 | 0.0 | -0.1 | 0.1 | 93.8 | 7.25 | 1.5 | 1.0 | 0.7 | 0.0 | 0.7 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 2.10 | 0.48 |
| | 9 | 4 | 3 | 9 | 2 | 6 | 2 | 2 | 5 | 8 | 8 | 6 | 7 | 0 | 1 | 3 | 1 | 2 | 1 | 1 | 0 | 8 | 18 | 1 |
| | | 8 | | 5 | 9 | 0 | 1 | 4 | 0 | 0 | | 7 | | 2 | | 6 | | 9 | | 0 | | 2 | | |
| Mixed | -0.3 | 0.1 | -0.1 | 0.1 | -0.1 | 0.0 | -0.0 | 0.0 | -0.1 | 0.1 | 83.4 | 5.64 | -0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.88 | 0.40 |
| rino | 3.2 | 0.2 | 1.6 | 0.6 | 1.5 | 0.4 | 0.1 | 0.1 | 2.7 | 0.7 | .67 | 5.04 | 0.4 | 0.3 | 0.8 | 0.3 | 0.0 | 0.3 | 0.0 | 0.1 | 0.3 | 0.5 | .78 | .47 |
| | 5 | 2 | 3 | 6 | 0 | 1 | 9 | 3 | 5 | 5 | | 7 | 2 | 4 | | 1 | | 7 | | 5 | | 1 | | |
| White | 0.1 | 0.1 | -0.1 | 0.1 | -0.0 | 0.0 | 0.0 | 0.0 | -0.1 | 0.1 | 62.7 | 7.66 | 0.8 | 0.0 | 0.6 | 0.0 | 0.6 | 0.0 | 0.0 | 0.0 | 0.8 | 0.0 | 66.53 | |
| Suffolk | 1.9 | 0.2 | 2.1 | 0.3 | 3.5 | 0.4 | 1.5 | 0.1 | 5.4 | 0.4 | 4.43 | 2.70 | 3.4 | 0.4 | 7.2 | 0.3 | 3.8 | 0.1 | 1.3 | 0.1 | 4.3 | 0.2 | 3.27 | 2.72 |
| folk | | 7 | 5 | 3 | 8 | 6 | | 8 | 6 | 0 | | 0 | | 0 | | 1 | | 3 | | 5 | | 9 | | |
| **LOW** | | | | | | | | | | | | | | | | | | | | | | | | |
| Dorset | 0.7 | 0.1 | 0.0 | 0.0 | -0.1 | 0.0 | 0.1 | 0.0 | 2.2 | 0.2 | 61.9 | 5.61 | 0.6 | 0.0 | 0.6 | 0.0 | 1.0 | 0.1 | 0.3 | 0.0 | 1.3 | 0.1 | 61.85 | |
| | 5 | 3 | 0 | 0 | 2 | 2 | 3 | 1 | 3 | 3 | 2 | 3 | 7 | 6 | 7 | 6 | 0 | 0 | 3 | 3 | 3 | 1 | 2 | 8 |
| | | 8 | | 0 | 5 | 5 | | 3 | | 2 | | 6 | | 7 | | 7 | | 0 | | 3 | | 7 | | |
| Mixed | 2.0 | 0.0 | 1.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.0 | 40.5 | -1.8 | 1.3 | -0.2 | 2.0 | -0.2 | 0.0 | 0.0 | -0.3 | 0.0 | 40.62 | | | |
| rino | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 8 | 0 | .83 | 3.12 | 1.0 | 0.0 | 0.7 | 0.0 | 1.5 | 0.0 | 0.0 | 0.0 | 0.8 | 0.0 | | |
| | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 1 | 2 | 6 | 2 | 3 | 7 | 1 | 5 | 1 | 0 | 0 | 3 | 3 | 2 | 7 |
| | | 0 | | 0 | | 3 | | 0 | | 7 | | 7 | 5 | 1 | 5 | 4 | 0 | 8 | | 0 | 8 | 8 | | |
| White | -0.2 | 0.2 | -0.1 | 0.1 | -0.1 | 0.1 | -0.0 | 0.0 | -0.2 | 0.2 | 20.6 | -1.9 | 1.0 | -0.1 | 1.0 | -0.1 | 0.0 | 0.0 | -0.1 | 0.0 | 20.48 | | | |
| ite | 0.1 | 0.1 | 1.0 | 0.1 | 1.0 | 0.1 | 0.0 | 0.0 | 2.0 | 0.1 | .49 | 9.16 | 1.0 | 0.1 | 1.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 1.0 | 0.1 | .49 | .52 |
| Suffolk | 7 | 4 | 2 | 7 | 5 | 8 | 3 | 3 | 0 | 3 | 1 | 2 | 5 | 5 | 0 | 3 | 2 | 9 | 1 | 3 | 5 | 5 | 1 | 8 |
| folk | 5 | 3 | 5 | 0 | 0 | 5 | 8 | 8 | 0 | 9 | | 1 | 0 | 5 | 0 | 5 | 5 | 5 | 3 | 1 | 0 | 1 | | |
| **MEDIUM** | | | | | | | | | | | | | | | | | | | | | | | | |
| Black | 0.3 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 | 0.1 | 0.0 | 0.5 | 0.0 | 13.4 | 5.96 | 0.6 | 0.0 | 0.0 | 0.1 | 0.3 | 0.0 | 0.0 | 0.0 | 0.8 | 0.0 | 15.96 | |
| Suffolk | 0.3 | 0.3 | 0.0 | 0.0 | 0.7 | 0.3 | 0.7 | 0.1 | 0.0 | 0.2 | 69.0 | 7.06 | 0.7 | 0.3 | 0.0 | 0.5 | 0.3 | 0.3 | 0.0 | 0.0 | 0.3 | 0.7 | 06.2 | |
| folk | | 3 | | 0 | | 3 | | 7 | | 9 | | 6 | | 3 | | 8 | | 3 | | 0 | | 3 | | |
| Dorset | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 13.2 | 0.48 | 0.0 | 0.0 | -0.0 | 0.0 | 0.0 | 0.0 | -0.0 | 0.0 | 0.0 | 0.0 | 94.50 | |
| | 4 | 2 | 7 | 3 | 7 | 3 | 7 | 1 | 7 | 3 | 73.2 | 9.29 | 2 | 8 | 5 | 7 | 9 | 4 | 0 | 1 | 6 | 7 | 0 | 2 |
| | | 0 | | 1 | | 3 | | 1 | | 1 | | 6 | | 1 | 7 | 5 | | 7 | 7 | 3 | | 2 | | |
| Mixed | -0.1 | 0.1 | -0.1 | 0.1 | -0.1 | 0.1 | 0.0 | 0.0 | -0.1 | 0.1 | 71.3 | -1.3 | -0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 11.76 | |
| rino | 1.9 | 0.4 | 0.6 | 0.0 | 1.5 | 0.2 | 0.0 | 0.1 | 0.3 | 0.3 | 3.99 | 5.41 | 1.4 | 0.8 | 0.4 | 0.7 | 0.9 | 0.2 | 0.7 | 0.1 | 0.9 | 0.3 | 33.6 | |
| | 9 | 8 | 3 | 7 | 0 | 2 | | 3 | 0 | 2 | | 1 | 3 | 1 | 3 | 8 | | 9 | | 3 | | 3 | | |
| White | -0.1 | 0.1 | -0.1 | 0.1 | 0.0 | 0.0 | -0.0 | 0.0 | -0.1 | 0.1 | 12.4 | 1.04 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -0.1 | 0.1 | 11.10 | |
| ite | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.0 | 0.0 | 0.7 | 0.1 | 1.44 | 9.14 | 0.4 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | 0.6 | 0.1 | 7.33 | |
| Suffolk | 7 | 7 | 9 | 5 | 9 | 4 | 0 | 3 | 2 | 3 | 55.7 | 7.30 | 3 | 6 | 3 | 2 | 9 | 8 | 4 | 0 | 6 | 1 | 33 | 82 |
| folk | 9 | 5 | 3 | 4 | | 2 | 7 | 0 | 3 | 3 | | 0 | | 1 | | 0 | | 4 | | 9 | 4 | 4 | | |
| **HIGH** | | | | | | | | | | | | | | | | | | | | | | | | |
| Black | -0.2 | 0.2 | -0.1 | 0.0 | -0.1 | 0.1 | 0.0 | 0.0 | -0.2 | 0.2 | 75.6 | 1.06 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 77.70 | |
| Suffolk | 0.3 | 0.0 | 0.5 | 0.8 | 0.5 | 0.1 | 0.0 | 0.3 | 0.3 | 0.2 | 1.50 | 5.00 | 0.0 | 0.5 | 0.0 | 0.0 | 0.7 | 0.6 | 0.0 | 0.0 | 0.7 | 0.1 | 5.52 | |
| folk | 8 | 1 | 0 | 7 | 0 | 9 | | 5 | 8 | 6 | | 1 | | 8 | | 0 | | 7 | | 0 | | 7 | | |
| Dorset | -0.1 | 0.1 | -0.0 | 0.0 | -0.1 | 0.1 | -0.0 | 0.0 | -0.1 | 0.0 | 5.88 | -1.8 | 0.1 | 0.0 | -0.1 | 0.0 | 0.0 | 0.1 | -0.1 | 0.0 | 0.0 | 0.1 | 90.95 | |
| | 1.3 | 0.3 | 1.1 | 0.7 | 1.1 | 0.6 | 0.1 | 0.2 | 2.1 | 0.9 | 10.6 | 8.85 | 1.1 | 0.5 | 1.9 | 0.5 | 0.8 | 0.3 | 0.2 | 0.1 | 1.9 | 0.2 | .82 | .04 |
| | 8 | 6 | 3 | 4 | 3 | 1 | 3 | 3 | 9 | 5 | | 3 | 0 | 8 | | 3 | 8 | 4 | 5 | 6 | | 9 | | |
| Mixed | 1.6 | 0.0 | -0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 11.5 | 0.07 | 0.0 | 0.0 | -0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 11.59 | |
| rino | 6 | 0.0 | 0.0 | 0.0 | 0 | 0.1 | 1 | 0.0 | 0 | 0.0 | 0.0 | 4.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0.2 | 2 | 0.2 | 2 | 0.5 | .8 | |
| | 7 | 4 | 3 | 5 | 0 | 6 | 7 | 1 | 0 | 5 | 20.5 | 1 | 5 | 1 | 8 | 0 | 5 | 1 | 1 | 9 | 6 | 31 | 7 | |
| | | 2 | 3 | 6 | | 3 | | 1 | | 3 | | 2 | | 7 | 4 | 3 | | 8 | | 0 | | 4 | | |
| White | -0.1 | 0.1 | -0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.1 | 10.5 | 1.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 10.44 | |
| ite | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 1 | 0.0 | 1 | 0.0 | 5.2 | 2.8 | 0.8 | 0.0 | 0.8 | 0.0 | 0.0 | 0.0 | 0 | 0.0 | 1 | 0.0 | 2.04 | .12 |
| Suffolk | 3 | 3 | 7 | 9 | 1 | 6 | 4 | 0 | 4 | 2 | 10.9 | 3 | 4 | 3 | 3 | 5 | 4 | 8 | 0 | 7 | 4 | 04 | 2 | |
| folk | 6 | 0 | 1 | 2 | 4 | 7 | | 9 | | 2 | | 6 | | 8 | | 1 | 0 | 3 | | 8 | | 6 | | |

[1] Change in (Δ): chest girth (CG), wither height (WH), body length (BL), body condition score (BCS), liveweight (BWT), average daily liveweight gain (ADG)

**S4.7** *Spirulina* supplementation level and plane of nutrition interactions on change in liveweight, body conformation and growth [1]

| | Ewe | | | | | | | | | | | | Wether | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ΔCG | | ΔWH | | ΔBL | | ΔBCS | | ΔBWT | | ADG | | ΔCG | | ΔWH | | ΔBL | | ΔBCS | | ΔBWT | | ADG | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| **High-plane** | | | | | | | | | | | | | | | | | | | | | | | | |
| Black Suffolk | 0.15 | 0.76 | 0.00 | 0.39 | 0.00 | 0.47 | 0.05 | 0.14 | -0.25 | 1.02 | 121.77 | 31.98 | 0.80 | 0.33 | 0.15 | 0.64 | 0.25 | 0.34 | -0.05 | 0.12 | 1.35 | 0.42 | 140.14 | 48.48 |
| Dorset | -0.23 | 0.39 | -0.27 | 0.71 | -0.45 | 0.52 | -0.09 | 0.16 | -0.12 | 1.92 | 147.14 | 32.72 | -0.20 | 0.71 | -0.26 | 0.62 | -0.04 | 0.99 | -0.05 | 0.58 | 0.07 | 0.69 | 131.97 | 36.23 |
| Merino | -1.86 | 0.46 | -0.81 | 0.88 | -1.05 | 0.24 | -0.91 | 0.13 | -1.54 | 4.42 | 259.25 | 25.70 | -0.55 | 1.08 | 0.05 | 0.59 | 0.00 | 0.48 | -0.11 | 0.09 | 0.05 | 0.89 | 38.43 | 4.03 |
| White Suffolk | -1.29 | 0.28 | -1.47 | 1.14 | -0.57 | 0.53 | -1.71 | 0.15 | -5.02 | 2.21 | 78.70 | 4.14 | -0.36 | 0.76 | -1.11 | 0.26 | -0.17 | 0.07 | -0.26 | 0.96 | -0.71 | 0.01 | 98.07 | 0.01 |
| **Low-plane** | | | | | | | | | | | | | | | | | | | | | | | | |
| Dorset | 0.36 | 0.92 | 0.29 | 0.42 | -0.21 | 0.98 | 0.00 | 0.14 | 0.32 | 0.89 | 151.35 | 41.18 | 0.43 | 0.84 | 0.50 | 0.23 | 0.29 | 0.76 | 0.04 | 0.14 | 0.36 | 0.69 | 63.78 | 4.14 |
| Merino | 0.07 | 0.84 | -0.59 | 0.93 | -0.22 | 0.65 | 0.00 | 0.07 | -0.96 | 0.82 | 452.75 | 50.57 | -0.52 | 0.70 | -0.01 | 0.72 | -0.04 | 0.67 | -0.01 | 0.14 | 0.03 | 0.46 | 56.41 | 3.37 |
| White Suffolk | 0.43 | 0.84 | 0.01 | 0.54 | -0.14 | 0.55 | 0.14 | 0.16 | 0.35 | 0.78 | 351.75 | 42.58 | 0.57 | 0.63 | 0.14 | 0.43 | 0.29 | 0.52 | 0.04 | 0.11 | 0.36 | 0.51 | 35.71 | 5.14 |

[1] Change in (Δ): chest girth (CG), wither height (WH), body length (BL), body condition score (BCS), liveweight (BWT), average daily liveweight gain (ADG)

## Chapter 5: Liveweight, body conformation and growth with high plane of nutrition

**S5.1** *Spirulina* supplementation level and sex interactions on liveweight, body conformation and growth under high plane of nutrition [1]

| | *Units* | CONTROL | | | | MEDIUM | | | | HIGH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| CG | cm | 92.41 | 0.75 | 97.58 | 0.82 | 96.59 | 1.13 | 94.68 | 0.60 | 95.68 | 0.87 | 96.48 | 1.06 |
| WH | cm | 62.13 | 0.43 | 63.59 | 0.50 | 62.34 | 0.54 | 63.05 | 0.47 | 62.73 | 0.38 | 63.46 | 0.53 |
| BL | cm | 64.50 | 0.59 | 66.81 | 0.58 | 66.80 | 0.75 | 66.38 | 0.44 | 67.15 | 0.55 | 66.36 | 0.44 |
| BCS | 1-5 | 3.13 | 0.05 | 3.31 | 0.08 | 3.34 | 0.07 | 3.26 | 0.05 | 3.41 | 0.06 | 3.43 | 0.09 |
| BWT | kg | 37.88 | 0.90 | 43.39 | 1.00 | 42.06 | 1.16 | 41.83 | 0.86 | 40.43 | 0.78 | 41.13 | 0.81 |
| ADG | kg/day | 0.13 | 0.03 | 0.17 | 0.04 | 0.13 | 0.03 | 0.14 | 0.05 | 0.13 | 0.03 | 0.12 | 0.03 |

[1] Chest girth (CG), withers height (WH), body length (BL), body condition score (BCS), body weight (BWT), and average daily weight gain (ADG)

## Chapter 6: Haematological metabolites

### **S6.1** Sire breed and sex interactions on haematological metabolite contentrations [1]

| | *Units* | Black Suffolk | | | | Dorset | | | | Merino | | | | White Suffolk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| CK | UI | 256.83 | 33.81 | 294.67 | 53.25 | 393.60 | 69.68 | 280.83 | 23.64 | 213.17 | 20.49 | 284.33 | 9.45 | 289.83 | 37.52 | 298.83 | 37.58 |
| AST | UI | 119.50 | 16.04 | 118.40 | 14.23 | 133.00 | 15.83 | 118.00 | 12.54 | 159.00 | 16.82 | 118.50 | 3.47 | 131.00 | 16.89 | 107.17 | 8.20 |
| GLDH | UI | 32.50 | 14.18 | 21.80 | 10.06 | 15.83 | 5.68 | 23.00 | 11.47 | 19.17 | 7.38 | 16.50 | 4.64 | 27.17 | 15.45 | 16.17 | 4.91 |
| GGT | UI | 86.00 | 2.53 | 68.00 | 2.84 | 82.33 | 2.28 | 78.00 | 4.28 | 64.40 | 5.50 | 60.83 | 2.88 | 74.00 | 4.83 | 69.17 | 4.50 |
| Total Bilirubin | µmol/L | 2.90 | 0.13 | 3.05 | 0.29 | 3.83 | 0.47 | 3.35 | 0.28 | 2.85 | 0.09 | 3.37 | 0.29 | 2.92 | 0.30 | 2.92 | 0.09 |
| Creatinine | µmol/L | 58.00 | 2.53 | 63.67 | 1.84 | 58.17 | 4.24 | 59.50 | 2.81 | 55.83 | 2.09 | 61.83 | 1.58 | 56.33 | 2.86 | 60.67 | 2.39 |
| Urea | mmol/L | 7.05 | 0.55 | 7.33 | 0.67 | 7.88 | 0.36 | 7.12 | 0.29 | 7.05 | 0.65 | 9.30 | 0.38 | 8.52 | 0.39 | 8.42 | 0.44 |
| Calcium | nmol/L | 2.51 | 0.05 | 2.50 | 0.06 | 2.49 | 0.06 | 2.57 | 0.06 | 2.52 | 0.03 | 2.57 | 0.03 | 2.48 | 0.06 | 2.53 | 0.07 |
| Magnesium | mmol/L | 0.94 | 0.02 | 0.94 | 0.04 | 0.91 | 0.02 | 1.01 | 0.03 | 0.93 | 0.03 | 0.91 | 0.03 | 0.93 | 0.03 | 0.92 | 0.03 |
| Phosphate | mmol/L | 1.90 | 0.12 | 2.01 | 0.12 | 1.93 | 0.10 | 2.12 | 0.13 | 1.83 | 0.10 | 1.79 | 0.09 | 2.13 | 0.05 | 2.13 | 0.08 |
| Sodium | mmol/L | 142.67 | 0.42 | 142.17 | 0.48 | 142.00 | 0.45 | 142.17 | 0.48 | 142.67 | 0.42 | 141.50 | 0.34 | 142.67 | 0.67 | 142.00 | 0.45 |
| Potassium | mmol/L | 4.60 | 0.11 | 4.77 | 0.20 | 4.78 | 0.16 | 4.70 | 0.05 | 4.48 | 0.13 | 4.73 | 0.09 | 4.85 | 0.23 | 4.80 | 0.12 |
| Na/K Ratio | . | 31.00 | 0.77 | 30.33 | 1.12 | 29.83 | 1.05 | 30.33 | 0.33 | 32.17 | 0.79 | 30.17 | 0.60 | 29.67 | 1.23 | 29.67 | 0.71 |
| Chloride | mmol/L | 105.83 | 0.75 | 106.00 | 0.77 | 107.33 | 0.95 | 106.50 | 1.12 | 104.83 | 0.40 | 104.67 | 0.33 | 106.83 | 0.79 | 106.33 | 0.67 |
| Protein | mmol/L | 62.92 | 0.66 | 66.53 | 2.37 | 64.00 | 1.17 | 68.10 | 2.06 | 62.38 | 1.90 | 63.38 | 0.95 | 63.98 | 2.37 | 67.80 | 1.17 |
| Albumin | g/L | 35.52 | 0.52 | 36.93 | 0.42 | 36.05 | 0.81 | 36.72 | 0.64 | 33.78 | 1.02 | 34.13 | 0.79 | 36.42 | 1.13 | 37.27 | 0.52 |
| Globulin | g/L | 27.67 | 1.45 | 27.33 | 1.45 | 29.00 | 3.21 | 30.00 | 3.06 | 29.00 | 1.15 | 28.33 | 2.85 | 28.00 | 2.31 | 30.67 | 1.33 |
| A/G Ratio | g/L | 1.30 | 0.06 | 1.28 | 0.08 | 1.32 | 0.10 | 1.20 | 0.09 | 1.21 | 0.09 | 1.19 | 0.08 | 1.33 | 0.05 | 1.22 | 0.02 |
| BHB | mmol/L | 0.46 | 0.04 | 0.43 | 0.04 | 0.38 | 0.04 | 0.37 | 0.04 | 0.42 | 0.05 | 0.43 | 0.04 | 0.48 | 0.03 | 0.33 | 0.2 |
| Glucose | mmol/L | 3.98 | 0.16 | 3.87 | 0.10 | 4.70 | 0.36 | 3.88 | 0.11 | 3.95 | 0.26 | 3.95 | 0.20 | 3.85 | 0.12 | 3.92 | 0.07 |
| NEFA | . | 1.01 | 0.11 | 0.80 | 0.13 | 0.99 | 0.12 | 0.71 | 0.09 | 0.59 | 0.06 | 0.95 | 0.15 | 0.72 | 0.11 | 0.64 | 0.08 |
| Cortisol | nmol/L | 57.67 | 15.06 | 94.50 | 23.64 | 77.00 | 23.72 | 46.67 | 14.87 | 51.83 | 11.00 | 47.33 | 9.48 | 31.00 | 1.75 | 49.17 | 12.89 |

[1] Creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), beta-hydroxybutyrate (BHB), albumin/globulin ratio (A/G Ratio), non esterfied fatty acids (NEFA)

**S6.2** *Spirulina* supplementation level and sex interactions on haematological metabolite concentrations [1]

| | *Units* | CONTROL | | | | MEDIUM | | | | HIGH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| CK | UI | 308.63 | 54.03 | 286.25 | 36.16 | 229.00 | 19.10 | 267.88 | 21.87 | 306.88 | 35.86 | 314.88 | 29.43 |
| AST | UI | 109.88 | 3.74 | 126.00 | 9.27 | 141.88 | 16.69 | 118.25 | 7.13 | 155.13 | 14.79 | 100.00 | 5.80 |
| GLDH | UI | 10.88 | 2.22 | 20.25 | 6.44 | 30.88 | 12.29 | 20.75 | 9.04 | 29.25 | 10.46 | 16.43 | 3.72 |
| GGT | UI | 84.57 | 2.51 | 74.88 | 3.33 | 77.38 | 3.94 | 64.25 | 3.18 | 70.63 | 4.81 | 67.88 | 3.98 |
| Total Bilirubin | µmol/L | 3.14 | 0.33 | 3.09 | 0.26 | 3.26 | 0.27 | 3.29 | 0.25 | 2.98 | 0.25 | 3.14 | 0.14 |
| Creatinine | µmol/L | 55.13 | 2.72 | 59.25 | 1.60 | 59.50 | 2.93 | 64.00 | 1.64 | 56.63 | 1.68 | 61.00 | 2.14 |
| Urea | mmol/L | 7.62 | 0.57 | 7.81 | 0.30 | 7.81 | 0.45 | 8.16 | 0.69 | 7.44 | 0.41 | 8.15 | 0.47 |
| Calcium | nmol/L | 2.46 | 0.03 | 2.56 | 0.05 | 2.55 | 0.03 | 2.48 | 0.03 | 2.49 | 0.06 | 2.59 | 0.05 |
| Magnesium | mmol/L | 0.91 | 0.02 | 0.99 | 0.03 | 0.97 | 0.02 | 0.92 | 0.03 | 0.90 | 0.02 | 0.93 | 0.02 |
| Phosphate | mmol/L | 2.00 | 0.11 | 2.04 | 0.08 | 1.87 | 0.07 | 2.00 | 0.10 | 1.98 | 0.09 | 2.00 | 0.12 |
| Sodium | mmol/L | 142.50 | 0.42 | 141.63 | 0.46 | 142.88 | 0.48 | 142.00 | 0.38 | 142.13 | 0.35 | 142.25 | 0.25 |
| Potassium | mmol/L | 4.69 | 0.14 | 4.91 | 0.12 | 4.73 | 0.20 | 4.71 | 0.09 | 4.63 | 0.08 | 4.63 | 0.07 |
| Na/K Ratio | . | 30.50 | 0.85 | 29.13 | 0.64 | 30.63 | 1.15 | 30.38 | 0.60 | 30.88 | 0.67 | 30.88 | 0.48 |
| Chloride | mmol/L | 106.38 | 0.46 | 105.75 | 0.56 | 106.38 | 0.91 | 105.25 | 0.62 | 105.88 | 0.74 | 106.63 | 0.82 |
| Protein | mmol/L | 61.69 | 0.94 | 67.24 | 1.65 | 65.13 | 1.75 | 67.04 | 1.83 | 63.15 | 1.15 | 65.09 | 1.24 |
| Albumin | g/L | 34.88 | 0.74 | 36.19 | 0.86 | 36.20 | 0.98 | 36.40 | 0.49 | 35.25 | 0.73 | 36.20 | 0.69 |
| Globulin | g/L | 25.25 | 1.31 | 29.75 | 1.93 | 30.75 | 1.49 | 28.75 | 1.49 | 29.25 | 0.75 | 28.75 | 2.50 |
| A/G Ratio | g/L | 1.32 | 0.07 | 1.18 | 0.06 | 1.27 | 0.06 | 1.21 | 0.05 | 1.28 | 0.07 | 1.27 | 0.07 |
| BHB | mmol/L | 0.39 | 0.04 | 0.36 | 0.03 | 0.48 | 0.02 | 0.37 | 0.04 | 0.44 | 0.04 | 0.43 | 0.03 |
| Glucose | mmol/L | 3.71 | 0.11 | 3.90 | 0.14 | 4.19 | 0.21 | 3.89 | 0.10 | 4.46 | 0.28 | 3.93 | 0.08 |
| NEFA | . | 0.80 | 0.09 | 0.85 | 0.11 | 0.89 | 0.13 | 0.64 | 0.11 | 0.80 | 0.10 | 0.83 | 0.08 |
| Cortisol | nmol/L | 59.00 | 18.16 | 66.75 | 17.90 | 51.00 | 10.58 | 60.13 | 15.77 | 53.13 | 12.55 | 51.38 | 12.04 |

[1] Creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), beta-hydroxybutyrate (BHB), albumin/globulin ratio (A/G Ratio), non esterfied fatty acids (NEFA)

**S6.3** *Spirulina* supplementation level and sire breed interactions on haematological metabolite concentrations [1]

| | *Units* | CONTROL | | | | | | | | MEDIUM | | | | | | | | HIGH | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Black Suffolk | | Dorset | | Merino | | White Suffolk | | Black Suffolk | | Dorset | | Merino | | White Suffolk | | Black Suffolk | | Dorset | | Merino | | White Suffolk | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| CK | UI | 314.25 | 64.42 | 387.75 | 75.81 | 211.25 | 25.55 | 276.50 | 63.19 | 188.25 | 21.49 | 269.67 | 41.53 | 275.25 | 26.11 | 274.75 | 5.25 | 328.75 | 38.46 | 323.25 | 67.19 | 257.75 | 22.88 | 337.68 | 47.68 |
| AST | UI | 1118.75 | 17.49 | 1127.25 | 9.60 | 1199.75 | 4.15 | 1006.00 | 7.72 | 1083.25 | 9.06 | 1373.00 | 22.87 | 1399.50 | 13.28 | 1355.00 | 26.96 | 1336.75 | 31.65 | 1112.25 | 18.88 | 1527.00 | 26.87 | 1157.50 | 9.29 |
| GLDH | UI | 22.25 | 12.10 | 12.75 | 2.87 | 9.50 | 3.77 | 17.75 | 6.73 | 23.75 | 12.13 | 27.75 | 17.45 | 18.00 | 8.30 | 33.75 | 23.57 | 40.00 | 26.59 | 12.75 | 8.43 | 26.00 | 7.95 | 13.50 | 3.23 |
| GGT | UI | 77.50 | 6.34 | 83.50 | 3.01 | 72.00 | 5.51 | 82.75 | 3.77 | 76.75 | 7.88 | 74.75 | 5.96 | 60.25 | 4.35 | 71.50 | 2.72 | 76.75 | 3.97 | 82.25 | 1.97 | 75.00 | 2.72 | 60.50 | 3.28 |
| Total Bilirubin | μmol/L | 2.60 | 0.11 | 3.75 | 0.52 | 3.08 | 0.41 | 3.03 | 0.39 | 3.33 | 0.35 | 3.65 | 0.59 | 3.03 | 0.26 | 3.10 | 0.14 | 3.00 | 0.15 | 3.38 | 0.42 | 2.23 | 0.24 | 2.63 | 0.20 |
| Creatinine | μmol/L | 58.75 | 3.54 | 54.00 | 4.60 | 59.50 | 1.55 | 56.50 | 3.12 | 63.00 | 3.49 | 67.00 | 1.22 | 54.50 | 2.72 | 62.50 | 3.30 | 60.75 | 2.17 | 55.50 | 2.96 | 62.50 | 2.33 | 56.50 | 3.28 |
| Urea | mmol/L | 6.95 | 0.69 | 7.23 | 0.36 | 8.20 | 0.75 | 8.50 | 0.44 | 7.42 | 1.00 | 8.00 | 0.48 | 8.11 | 1.15 | 8.50 | 0.53 | 7.44 | 0.44 | 7.24 | 0.41 | 8.01 | 0.84 | 8.44 | 0.63 |
| Calcium | mmol/L | 2.56 | 0.07 | 2.51 | 0.10 | 2.54 | 0.43 | 2.43 | 0.03 | 2.50 | 0.19 | 2.50 | 0.04 | 2.50 | 0.12 | 2.55 | 0.05 | 2.44 | 0.44 | 2.50 | 0.07 | 2.50 | 0.79 | 2.51 | 0.42 |
| Magnesium | mmol/L | 0.96 | 0.04 | 0.98 | 0.06 | 0.91 | 0.04 | 0.95 | 0.04 | 0.94 | 0.04 | 0.97 | 0.02 | 0.93 | 0.04 | 0.93 | 0.06 | 0.92 | 0.03 | 0.93 | 0.03 | 0.91 | 0.03 | 0.91 | 0.03 |
| Phosphate | mmol/L | 1.93 | 0.15 | 2.03 | 0.12 | 1.86 | 0.10 | 2.26 | 0.08 | 1.82 | 0.25 | 1.94 | 0.15 | 1.89 | 0.11 | 2.08 | 0.05 | 2.21 | 0.23 | 2.11 | 0.17 | 1.77 | 0.11 | 2.06 | 0.06 |
| Sodium | mmol/L | 143.00 | 0.71 | 141.50 | 0.29 | 142.25 | 0.75 | 141.50 | 0.65 | 142.00 | 0.41 | 142.75 | 0.75 | 141.75 | 0.48 | 143.25 | 0.75 | 142.25 | 0.48 | 142.00 | 0.41 | 142.25 | 0.48 | 142.25 | 0.48 |
| Potassium | mmol/L | 4.88 | 0.27 | 4.73 | 0.24 | 4.88 | 0.06 | 4.73 | 0.18 | 4.55 | 0.18 | 4.70 | 0.09 | 4.55 | 0.19 | 5.05 | 0.29 | 4.63 | 0.09 | 4.75 | 0.09 | 4.30 | 0.02 | 4.70 | 0.15 |
| Na/K Ratio | . | 29.50 | 1.44 | 30.25 | 1.49 | 29.50 | 0.50 | 30.00 | 1.08 | 31.55 | 1.19 | 30.63 | 0.61 | 31.75 | 1.31 | 28.54 | 1.44 | 31.00 | 0.71 | 29.75 | 0.63 | 32.55 | 0.25 | 30.54 | 1.04 |
| Chloride | mmol/L | 107.75 | 0.75 | 106.25 | 0.25 | 105.75 | 0.48 | 105.00 | 0.41 | 104.59 | 2.98 | 107.08 | 1.68 | 104.25 | 0.25 | 107.50 | 0.50 | 105.55 | 0.65 | 107.55 | 1.55 | 104.78 | 0.48 | 107.23 | 0.03 |
| Protein | mmol/L | 63.55 | 1.41 | 66.60 | 3.63 | 61.83 | 0.41 | 65.88 | 2.64 | 68.32 | 3.06 | 67.82 | 1.10 | 60.88 | 2.23 | 67.35 | 1.74 | 62.30 | 0.54 | 63.73 | 0.91 | 66.00 | 1.18 | 64.45 | 3.11 |
| Albumin | g/L | 36.00 | 0.97 | 36.40 | 0.61 | 32.88 | 1.18 | 36.85 | 0.77 | 36.38 | 0.67 | 35.58 | 1.25 | 35.45 | 1.21 | 37.80 | 1.00 | 36.30 | 0.44 | 37.18 | 0.67 | 33.55 | 0.41 | 35.88 | 1.36 |
| Globulin | g/L | 26.00 | 1.00 | 28.50 | 5.50 | 27.50 | 1.50 | 28.00 | 4.00 | 30.00 | 0.00 | 30.00 | 1.00 | 26.00 | 1.00 | 30.00 | 2.00 | 26.50 | 1.50 | 27.00 | 3.00 | 32.50 | 1.50 | 30.00 | 2.00 |
| A/G Ratio | g/L | 1.31 | 0.06 | 1.26 | 0.15 | 1.14 | 0.08 | 1.28 | 0.07 | 1.16 | 0.09 | 1.15 | 0.04 | 1.40 | 0.07 | 1.28 | 0.01 | 1.40 | 0.05 | 1.41 | 0.08 | 1.04 | 0.04 | 1.27 | 0.07 |
| BHB | mmol/L | 0.41 | 0.05 | 0.30 | 0.00 | 0.33 | 0.02 | 0.46 | 0.05 | 0.46 | 0.04 | 0.40 | 0.05 | 0.49 | 0.05 | 0.37 | 0.04 | 0.46 | 0.06 | 0.43 | 0.05 | 0.45 | 0.03 | 0.39 | 0.07 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose | mmol/L | 3.90 | 0.15 | 3.73 | 0.09 | 3.78 | 0.35 | 3.83 | 0.03 | 3.93 | 0.20 | 4.35 | 0.23 | 4.03 | 0.34 | 3.85 | 0.16 | 3.95 | 0.17 | 4.80 | 0.51 | 4.05 | 0.12 | 3.98 | 0.13 |
| NEFA | . | 0.928 | 0.213 | 0.859 | 0.053 | 0.684 | 0.17 | 0.853 | 0.11 | 0.913 | 0.16 | 0.804 | 0.25 | 0.755 | 0.181 | 0.573 | 0.11 | 0.866 | 0.121 | 0.918 | 0.082 | 0.885 | 0.161 | 0.625 | 0.091 |
| Cortisol | nmol/L | 5.50 | 1.11 | 3.00 | 3.20 | 0.50 | 4.21 | 2.50 | 4.50 | 7.75 | 9.34 | 1.75 | 8.37 | 7.00 | 3.92 | 5.75 | 6.79 | 5.00 | 5.86 | 0.75 | 2.75 | 1.25 | 6.54 | 2.00 | 9.14 |

[1] Creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), beta-hydroxybutyrate (BHB), albumin/globulin ratio (A/G Ratio), non esterfied fatty acids (NEFA)

## Chapter 7: Feed intake with low plane of nutrition

### S7.1 *Spirulina* supplementation level and sire breed interactions on feed intake models and specific growth rate [1]

| | CONTROL | | | | | | LOW | | | | | | MEDIUM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dorset | | Merino | | White Suffolk | | Dorset | | Merino | | White Suffolk | | Dorset | | Merino | | Wh | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Me | |
| DFI | 1.43 | 0.03 | 1.20 | 0.01 | 1.36 | 0.01 | 1.43 | 0.01 | 1.24 | 0.10 | 1.46 | 0.00 | 1.40 | 0.04 | 1.20 | 0.03 | 1. | |
| RFI | 64.98 | 0.40 | 67.15 | 2.07 | 68.15 | 1.57 | 62.47 | 2.32 | 68.85 | 5.54 | 60.43 | 4.35 | 63.41 | 0.86 | 67.58 | 0.66 | 66. | |
| RLG | 20.17 | 0.59 | 16.78 | 1.06 | 20.48 | 0.82 | 19.57 | 1.45 | 17.88 | 0.30 | 19.03 | 1.82 | 18.43 | 0.88 | 16.51 | 0.54 | 20. | |
| SDFI | 25.51 | 0.41 | 22.00 | 0.11 | 24.42 | 0.19 | 25.79 | 0.22 | 22.77 | 1.51 | 26.13 | 0.06 | 25.49 | 0.55 | 22.45 | 0.50 | 25. | |
| FCR | 19.54 | 4.26 | 39.23 | 0.23 | 35.44 | 8.56 | 37.53 | 9.73 | 20.18 | 1.62 | 28.52 | 0.08 | 14.19 | 2.49 | 20.22 | 3.90 | 16. | |
| $FCR_{met}$ | 3829.53 | 824.47 | 7894.61 | 39.42 | 7000.41 | 1699.42 | 7422.41 | 1914.73 | 4085.76 | 273.10 | 5629.31 | 13.51 | 2846.89 | 510.25 | 4156.18 | 782.79 | 3338. | |
| SGR | 7.65 | 1.53 | 3.06 | 0.00 | 4.08 | 1.02 | 4.08 | 1.02 | 6.12 | 0.00 | 5.10 | 0.00 | 10.20 | 2.04 | 6.12 | 1.02 | 8. | |

[1] Daily feed intake (DFI), residual feed intake model (RFI), residual liveweight gain model (RLG), standardised daily feed intake model (SDFI), feed conversion ratio (FCR), metabolic FCR ($FCR_{met}$), and specific growth rate (SGR)

### S7.2 *Spirulina* supplementation level and sex interactions on feed intake models and specific growth rate [1]

| | CONTROL | | | | LOW | | | | MEDIUM | | | | HIGH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| DFI | 1.31 | 0.06 | 1.35 | 0.07 | 1.41 | 0.04 | 1.34 | 0.10 | 1.30 | 0.04 | 1.37 | 0.10 | 1.34 | 0.07 | 1.33 | 0.10 |
| RFI | 65.68 | 0.46 | 67.84 | 1.64 | 61.39 | 2.69 | 66.44 | 4.19 | 65.65 | 1.56 | 65.99 | 1.18 | 64.73 | 3.66 | 68.22 | 2.63 |
| RLG | 18.32 | 1.30 | 19.97 | 1.07 | 18.60 | 1.22 | 19.05 | 0.90 | 17.87 | 0.58 | 19.02 | 1.68 | 18.37 | 0.25 | 19.64 | 1.16 |
| SDFI | 23.74 | 0.96 | 24.21 | 1.12 | 25.49 | 0.61 | 24.30 | 1.53 | 24.08 | 0.59 | 25.07 | 1.60 | 25.10 | 1.00 | 25.07 | 1.56 |
| FCR | 32.76 | 8.86 | 30.05 | 4.79 | 32.56 | 7.61 | 24.94 | 3.19 | 19.77 | 2.24 | 14.27 | 1.36 | 7.67 | 13.10 | 25.11 | 10.10 |
| $FCR_{met}$ | 6520.03 | 1774.32 | 5963.00 | 1003.05 | 6446.27 | 1492.20 | 4978.71 | 583.86 | 4013.71 | 475.99 | 2880.79 | 300.39 | 1579.31 | 2680.55 | 5167.33 | 2054.32 |
| SGR | 5.10 | 2.04 | 4.76 | 0.90 | 4.76 | 0.90 | 5.44 | 0.34 | 6.80 | 0.90 | 9.86 | 1.48 | 2.38 | 5.47 | 6.80 | 1.89 |

[1] Daily feed intake (DFI), residual feed intake model (RFI), residual liveweight gain model (RLG), standardised daily feed intake model (SDFI), feed conversion ratio (FCR), metabolic FCR ($FCR_{met}$), and specific growth rate (SGR)

### S7.3 Sire breed and sex interactions on feed intake models and specific growth rate [1]

| | Dorset | | | | Merino | | | | White Suffolk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe | | Wether | | Ewe | | Wether | | Ewe | | Wether | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| DFI | 1.40 | 0.02 | 1.42 | 0.01 | 1.24 | 0.03 | 1.16 | 0.02 | 1.39 | 0.03 | 1.45 | 0.03 |
| RFI | 63.82 | 0.74 | 63.51 | 1.13 | 66.79 | 1.84 | 71.32 | 1.52 | 62.47 | 2.74 | 66.54 | 1.10 |
| RLG | 19.26 | 0.73 | 19.50 | 0.55 | 17.12 | 0.52 | 17.38 | 0.49 | 18.50 | 0.53 | 21.37 | 0.20 |
| SDFI | 25.43 | 0.25 | 25.84 | 0.10 | 23.07 | 0.49 | 21.84 | 0.20 | 25.31 | 0.59 | 26.29 | 0.63 |
| FCR | 15.60 | 13.09 | 27.14 | 6.94 | 24.20 | 5.61 | 22.07 | 5.87 | 29.77 | 5.27 | 21.56 | 3.55 |
| $FCR_{met}$ | 3061.88 | 2612.43 | 5442.67 | 1441.65 | 4911.04 | 1111.66 | 4521.55 | 1150.55 | 5946.57 | 1023.92 | 4278.16 | 687.76 |
| SGR | 3.06 | 3.97 | 6.63 | 1.98 | 6.12 | 1.50 | 6.12 | 1.10 | 5.10 | 0.83 | 7.40 | 1.34 |

[1] Daily feed intake (DFI), residual feed intake model (RFI), residual liveweight gain model (RLG), standardised daily feed intake model (SDFI), feed conversion ratio (FCR), metabolic FCR ($FCR_{met}$), and specific growth rate (SGR)

## Chapter 8: Intramuscular fat percentage and fat melting points

### S8.1 *Spirulina* supplementation level interactions with sire breed, sex and plane of nutrition on intramuscular fat percentage and fat melting point [1]

| | | Sire breed | | | | | | | | Sex | | | | Plane of nutrition | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Spirulina level* | Black Suffolk | | Dorset | | Merino | | White Suffolk | | Ewe | | Wether | | Low- | | High- | |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| IMF | CONTROL | 1.48 | 0.23 | 2.37 | 0.41 | 3.45 | 1.38 | 2.33 | 0.26 | 2.55 | 0.23 | 2.07 | 0.36 | 2.58 | 0.36 | 2.05 | 0.27 |
| | LOW | . | . | 3.79 | 0.85 | 1.71 | 0.13 | 3.61 | 0.62 | 2.42 | 0.69 | 3.43 | 0.52 | 3.18 | 0.43 | . | . |
| | MEDIUM | 1.65 | 0.21 | 1.86 | 0.16 | 1.97 | 0.52 | 2.29 | 0.25 | 1.91 | 0.12 | 2.03 | 0.22 | 2.48 | 0.16 | 1.54 | 0.14 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HIGH | 1.65 | 0.21 | 2.71 | 0.51 | 2.12 | 0.58 | 2.41 | 0.61 | 3.24 | 0.54 | 1.69 | 0.21 | 3.63 | 0.50 | 1.41 | 0.11 |
| FMP | CONTROL | 40.57 | 0.45 | 42.96 | 0.78 | 46.25 | 0.06 | 43.61 | 0.39 | 43.29 | 0.62 | 43.07 | 0.54 | 42.52 | 0.49 | 43.89 | 0.62 |
| | LOW | . | . | 45.33 | 0.17 | 46.63 | 0.13 | 42.91 | 0.34 | 42.15 | 0.35 | 45.21 | 0.37 | 44.44 | 0.45 | . | . |
| | MEDIUM | 40.25 | 1.41 | 44.03 | 0.45 | 45.36 | 0.98 | 41.58 | 0.75 | 43.05 | 0.67 | 42.90 | 0.62 | 42.96 | 0.59 | 42.99 | 0.70 |
| | HIGH | 43.77 | 0.08 | 45.36 | 0.41 | 44.99 | 0.15 | 44.61 | 0.29 | 44.90 | 0.35 | 44.83 | 0.21 | 45.60 | 0.26 | 44.15 | 0.14 |

[1] Intramuscular fat percentage (IMF), subcutaneous fat melting point (FMP)

## **S8.2** *Spirulina* supplementation level and plane of nutrition interactions with sire breed on intramuscular fat percentage and fat melting point [1]

| | Black Suffolk | | | | Dorset | | | | Merino | | | | White Suffolk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IMF | | FMP | | IMF | | FMP | | IMF | | FMP | | IMF | | FMP | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| **CONTROL** | | | | | | | | | | | | | | | | |
| Low- | . | . | . | . | 1.97 | 0.48 | 40.75 | 0.38 | 3.45 | 1.38 | 46.25 | 0.06 | 2.76 | 0.38 | 42.43 | 0.08 |
| High- | 1.48 | 0.23 | 40.57 | 0.45 | 2.76 | 0.68 | 45.90 | 0.63 | . | . | . | . | 1.90 | 0.26 | 45.20 | 0.08 |
| **LOW** | | | | | | | | | | | | | | | | |
| Low- | . | . | . | . | 3.79 | 0.85 | 45.33 | 0.17 | 1.71 | 0.13 | 46.63 | 0.13 | 3.61 | 0.62 | 42.91 | 0.34 |
| **MEDIUM** | | | | | | | | | | | | | | | | |
| Low- | . | . | . | . | 3.94 | 0.70 | 46.44 | 0.35 | 3.25 | 0.66 | 44.70 | 0.12 | 3.51 | 1.06 | 45.21 | 0.41 |
| High- | 1.65 | 0.21 | 40.25 | 1.41 | 1.31 | 0.20 | 43.35 | 0.32 | 0.99 | 0.00 | 48.07 | 0.12 | 1.94 | 0.36 | 42.82 | 0.77 |
| **HIGH** | | | | | | | | | | | | | | | | |
| Low- | . | . | . | . | 3.94 | 0.70 | 46.44 | 0.35 | 3.25 | 0.66 | 44.70 | 0.12 | 3.51 | 1.06 | 45.21 | 0.41 |
| High- | 1.65 | 0.21 | 43.77 | 0.08 | 1.48 | 0.22 | 43.93 | 0.26 | 1.00 | 0.00 | 45.37 | 0.07 | 1.31 | 0.21 | 44.15 | 0.27 |

[1] Intramuscular fat percentage (IMF), subcutaneous fat melting point (FMP)

## **S8.3** *Spirulina* supplementation level and plane of nutrition interactions with sex on intramuscular fat percentage and subcutaneous fat melting point[1]

| | Ewe | | | | Wether | | | |
|---|---|---|---|---|---|---|---|---|
| | IMF | | FMP | | IMF | | FMP | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| **CONTROL** | | | | | | | | |
| Low- | 2.46 | 0.26 | 42.24 | 0.28 | 2.66 | 0.59 | 42.71 | 0.80 |
| High- | 1.90 | 0.26 | 45.20 | 0.08 | 1.48 | 0.33 | 43.54 | 0.67 |
| **LOW** | | | | | | | | |
| Low- | 2.42 | 0.69 | 42.15 | 0.35 | 3.43 | 0.52 | 45.21 | 0.37 |
| **MEDIUM** | | | | | | | | |
| Low- | 2.19 | 0.11 | 45.08 | 0.37 | 2.78 | 0.27 | 40.83 | 0.72 |
| High- | 1.63 | 0.16 | 40.33 | 0.85 | 1.48 | 0.22 | 44.98 | 0.56 |
| **HIGH** | | | | | | | | |
| Low- | 5.63 | 0.23 | 45.73 | 0.60 | 2.29 | 0.37 | 45.52 | 0.21 |
| High- | 1.65 | 0.17 | 44.17 | 0.22 | 1.23 | 0.13 | 44.14 | 0.22 |

[1] Intramuscular fat percentage (IMF), subcutaneous fat melting point (FMP)

## **S8.4** *Spirulina* supplementation level and sex interactions with sire breed on intramuscular fat percentage and subcutaneous fat melting point[1]

| | Black Suffolk | | | | Dorset | | | | Merino | | | | White Suffolk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IMF | | FMP | | IMF | | FMP | | IMF | | FMP | | IMF | | FMP | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| **CONTRO** | | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **L** | | | | | | | | | | | | | | | |
| Ewe | 1.99 | 0.01 | 40.23 | 0.92 | 3.27 | 0.40 | 44.07 | 1.16 | . | . | . | . | 2.11 | 0.17 | 43.83 | 0.48 |
| Wether | 0.97 | 0.02 | 40.90 | 0.21 | 1.46 | 0.51 | 41.84 | 0.95 | 3.45 | 1.38 | 46.25 | 0.06 | 2.54 | 0.49 | 43.40 | 0.66 |
| **LOW** | | | | | | | | | | | | | | | | |
| Ewe | . | . | . | . | . | . | . | . | . | . | . | . | 2.42 | 0.69 | 42.15 | 0.35 |
| Wether | . | . | . | . | 3.79 | 0.85 | 45.33 | 0.17 | 1.71 | 0.13 | 46.63 | 0.13 | 4.79 | 0.23 | 43.68 | 0.19 |
| **MEDIUM** | | | | | | | | | | | | | | | | |
| Ewe | 1.98 | 0.01 | 37.10 | 0.06 | 1.94 | 0.15 | 44.98 | 0.48 | . | . | . | . | 1.83 | 0.29 | 42.56 | 0.53 |
| Wether | 1.33 | 0.33 | 1.73 | 0.34 | 1.73 | 0.34 | 42.54 | 0.54 | 1.97 | 0.52 | 45.36 | 0.98 | 2.76 | 0.32 | 40.60 | 1.36 |
| **HIGH** | | | | | | | | | | | | | | | | |
| Ewe | 1.99 | 0.01 | 43.70 | 0.15 | 3.71 | 0.78 | 46.04 | 0.62 | . | . | . | . | 3.40 | 1.10 | 44.27 | 0.21 |
| Wether | 1.31 | 0.33 | 43.83 | 0.07 | 1.71 | 0.36 | 44.69 | 0.44 | 2.12 | 0.58 | 44.99 | 0.15 | 1.41 | 0.20 | 45.24 | 0.94 |

[1] Intramuscular fat percentage (IMF), subcutaneous fat melting point (FMP)

## Chapter 9: Fatty acid profile: plane of nutrition

**S9.1** Sire breed and sex interactions with plane of nutrition on subcutaneous adipose tissue fatty acid composition [1]

| (% total) | Black Suffolk | | Dorset | | | | Merino | | | | White Suffolk | | | | Ewe | | | | Wether | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High (*n* 6) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 2) | | Low (*n* 4) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 9) | | Low (*n* 8) | | High (*n* 11) | | Low (*n* 12) | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 2.5 | 0.6 | 2.7 | 0.1 | 2.2 | 0.5 | 3.6 | 1.2 | 3.1 | 0.2 | 2.7 | 0.2 | 2.8 | 0.5 | 2.7 | 0.2 | 2.5 | 0.5 | 2.7 | 0.3 | 2.7 | 0.3 |
| 15:0 | 0.7 | 0.1 | 0.7 | 0.0 | 0.6 | 0.1 | 0.7 | 0.1 | 0.7 | 0.2 | 0.6 | 0.1 | 0.7 | 0.1 | 0.7 | 0.0 | 0.6 | 0.1 | 0.7 | 0.1 | 0.6 | 0.1 |
| 16:1ω9c | 0.4 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 | 0.5 | 0.1 | 0.4 | 0.0 | 0.4 | 0.0 | 0.4 | 0.0 | 0.4 | 0.0 | 0.3 | 0.0 | 0.4 | 0.0 | 0.3 | 0.0 |
| 16:1ω7c | 1.3 | 0.1 | 1.3 | 0.1 | 0.9 | 0.3 | 1.0 | 0.2 | 0.5 | 0.3 | 1.5 | 0.2 | 1.2 | 0.3 | 1.3 | 0.1 | 1.0 | 0.3 | 1.3 | 0.1 | 0.9 | 0.2 |
| 16:0 | 24.1 | 0.5 | 23.3 | 0.5 | 23.8 | 1.3 | 22.2 | 1.0 | 25.5 | 0.9 | 23.5 | 0.6 | 24.2 | 0.8 | 23.3 | 0.4 | 24.4 | 1.5 | 23.7 | 0.4 | 24.2 | 0.5 |
| 17:0 | 2.5 | 0.2 | 1.9 | 0.1 | 1.8 | 0.1 | 1.8 | 0.1 | 2.1 | 0.2 | 1.8 | 0.2 | 1.8 | 0.1 | 2.0 | 0.1 | 1.9 | 0.1 | 2.0 | 0.1 | 1.9 | 0.1 |
| 18:2ω6 | 1.6 | 0.1 | 1.5 | 0.1 | 1.4 | 0.2 | 1.8 | 0.2 | 2.2 | 0.3 | 1.7 | 0.1 | 1.5 | 0.1 | 1.6 | 0.1 | 1.4 | 0.2 | 1.7 | 0.1 | 1.7 | 0.1 |
| 18:3ω3 | 1.4 | 0.1 | 1.6 | 0.1 | 1.1 | 0.1 | 1.5 | 0.0 | 1.7 | 0.2 | 1.7 | 0.2 | 1.2 | 0.1 | 1.5 | 0.1 | 1.1 | 0.1 | 1.6 | 0.1 | 1.4 | 0.1 |
| 18:1ω9 | 32.9 | 1.3 | 33.9 | 1.3 | 31.8 | 3.5 | 28.0 | 0.9 | 29.5 | 2.8 | 35.1 | 1.4 | 34.1 | 1.6 | 34.7 | 1.3 | 30.5 | 2.9 | 32.3 | 0.9 | 33.5 | 1.9 |
| 18:1ω7c | 1.6 | 0.1 | 1.4 | 0.0 | 1.0 | 0.2 | 1.2 | 0.0 | 1.2 | 0.1 | 1.4 | 0.0 | 1.2 | 0.1 | 1.5 | 0.1 | 1.0 | 0.1 | 1.4 | 0.1 | 1.2 | 0.0 |
| 18:1ω7t | 4.3 | 0.3 | 3.8 | 0.3 | 2.7 | 0.2 | 4.8 | 0.9 | 3.0 | 0.2 | 3.7 | 0.2 | 3.7 | 0.4 | 4.0 | 0.3 | 2.9 | 0.3 | 4.1 | 0.2 | 3.3 | 0.3 |
| 18:0 | 21.0 | 2.0 | 23.1 | 1.3 | 28.2 | 4.9 | 29.3 | 2.8 | 25.6 | 1.8 | 20.6 | 1.0 | 23.2 | 1.3 | 21.5 | 1.4 | 28.2 | 4.7 | 23.1 | 1.4 | 24.0 | 1.4 |
| 20:4ω6 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:5ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:3ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 20:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.4 | 0.3 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.2 | 0.1 |
| 22:6ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 |
| 22:5ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 23:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ∑SFA | 51.4 | 1.5 | 52.5 | 1.4 | 57.7 | 3.9 | 58.4 | 0.4 | 57.7 | 2.8 | 49.9 | 1.3 | 53.4 | 1.5 | 50.8 | 1.3 | 58.7 | 3.3 | 53.0 | 1.2 | 54.2 | 1.8 |
| ∑MUFA | 45.2 | 1.4 | 44.1 | 1.3 | 39.2 | 3.9 | 38.0 | 0.5 | 37.6 | 2.9 | 45.9 | 1.4 | 43.3 | 1.6 | 45.7 | 1.3 | 38.2 | 3.3 | 43.2 | 1.0 | 42.1 | 2.0 |
| ∑PUFA | 3.4 | 0.3 | 3.4 | 0.2 | 1.4 | 0.6 | 3.5 | 0.2 | 4.7 | 0.2 | 4.2 | 0.4 | 3.2 | 0.2 | 3.5 | 0.3 | 1.8 | 0.4 | 3.8 | 0.2 | 3.7 | 0.2 |
| ∑n-3 | 1.4 | 0.1 | 1.6 | 0.1 | 2.2 | 0.5 | 1.5 | 0.0 | 2.7 | 0.2 | 2.1 | 0.3 | 1.3 | 0.1 | 1.6 | 0.1 | 2.1 | 0.2 | 1.8 | 0.2 | 1.5 | 0.1 |
| ∑n-6 | 1.7 | 0.1 | 1.6 | 0.1 | 1.7 | 0.2 | 1.8 | 0.2 | 1.6 | 0.1 | 1.8 | 0.1 | 1.6 | 0.1 | 1.6 | 0.1 | 1.7 | 0.2 | 1.7 | 0.1 | 1.0 | 0.2 |
| ∑OtherFA | 5.6 | 0.4 | 4.5 | 0.2 | 3.7 | 0.5 | 3.6 | 0.4 | 3.9 | 0.3 | 4.9 | 0.2 | 3.7 | 0.2 | 4.9 | 0.2 | 3.7 | 0.5 | 4.8 | 0.3 | 3.8 | 0.2 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.2** Sire breed and sex interactions with plane of nutrition on heart tissue fatty acid composition [1]

| (% total) | Black Suffolk | | Dorset | | | | Merino | | | | White Suffolk | | | | Ewe | | | | Wether | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High (*n* 6) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 2) | | Low (*n* 4) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 9) | | Low (*n* 8) | | High (*n* 11) | | Low (*n* 12) | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 0.8 | 0.2 | 1.0 | 0.3 | 0.7 | 0.2 | 0.6 | 0.1 | 1.4 | 0.4 | 0.4 | 0.1 | 0.5 | 0.2 | 0.9 | 0.2 | 0.7 | 0.1 | 0.6 | 0.1 | 0.9 | 0.2 |
| 15:0 | 0.4 | 0.0 | 0.5 | 0.0 | 0.3 | 0.1 | 0.4 | 0.0 | 0.2 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 | 0.4 | 0.0 | 0.2 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 |
| 16:1ω9c | 0.2 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 |
| 16:1ω7c | 0.4 | 0.0 | 0.5 | 0.1 | 0.2 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 | 0.5 | 0.0 | 0.2 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 |
| 16:0 | 13.4 | 0.4 | 15.8 | 0.6 | 14.5 | 0.6 | 14.6 | 0.5 | 17.5 | 0.8 | 14.1 | 0.6 | 14.3 | 0.3 | 15.1 | 0.4 | 14.1 | 0.4 | 13.9 | 0.5 | 15.7 | 0.5 |
| 17:0 | 1.3 | 0.1 | 1.3 | 0.0 | 1.4 | 0.1 | 1.2 | 0.1 | 1.5 | 0.1 | 1.1 | 0.0 | 1.4 | 0.0 | 1.3 | 0.1 | 1.4 | 0.1 | 1.3 | 0.0 | 1.4 | 0.0 |
| 18:2ω6 | 16.1 | 1.6 | 11.2 | 1.2 | 16.5 | 1.3 | 17.4 | 1.8 | 17.5 | 3.2 | 15.1 | 1.9 | 19.7 | 1.5 | 13.3 | 1.8 | 17.1 | 1.5 | 15.1 | 1.2 | 18.1 | 1.5 |
| 18:3ω3 | 3.7 | 0.3 | 3.4 | 0.3 | 2.3 | 0.4 | 4.8 | 0.7 | 3.0 | 0.5 | 4.3 | 0.6 | 2.0 | 0.4 | 3.3 | 0.3 | 2.3 | 0.4 | 4.2 | 0.6 | 2.3 | 0.4 |
| 18:1ω9 | 19.0 | 1.7 | 20.3 | 1.6 | 19.2 | 0.8 | 20.2 | 0.3 | 20.1 | 1.5 | 17.5 | 1.0 | 18.7 | 0.6 | 20.4 | 2.2 | 18.9 | 0.7 | 17.9 | 0.8 | 19.4 | 0.7 |
| 18:1ω7c | 2.0 | 0.1 | 1.7 | 0.1 | 1.1 | 1.1 | 2.0 | 0.2 | 1.9 | 0.1 | 1.9 | 0.1 | 1.1 | 1.1 | 1.9 | 0.1 | 2.1 | 1.1 | 1.9 | 0.1 | 2.0 | 0.1 |
| 18:1ω7t | 2.6 | 0.2 | 2.7 | 0.3 | 1.6 | 0.2 | 2.6 | 0.2 | 1.9 | 0.2 | 2.3 | 0.2 | 1.6 | 0.2 | 2.7 | 0.2 | 1.5 | 0.2 | 2.4 | 0.1 | 1.8 | 0.2 |
| 18:0 | 21.1 | 0.7 | 24.4 | 1.4 | 20.4 | 1.8 | 19.9 | 1.2 | 20.2 | 2.7 | 21.4 | 0.8 | 18.5 | 1.6 | 23.4 | 1.1 | 19.1 | 1.5 | 21.0 | 0.7 | 19.9 | 1.5 |
| 20:4ω6 | 6.3 | 0.3 | 9.3 | 0.3 | 3.6 | 0.6 | 7.5 | 0.3 | 3.0 | 0.4 | 4.0 | 0.8 | 9.5 | 0.5 | 7.8 | 0.8 | 8.5 | 0.5 | 6.6 | 0.6 | 0.5 | 0.5 |
| 20:5ω3 | 3.2 | 0.3 | 3.1 | 0.1 | 1.8 | 0.2 | 2.8 | 0.2 | 1.5 | 0.3 | 3.1 | 0.1 | 2.0 | 0.2 | 2.8 | 0.2 | 1.5 | 0.3 | 3.2 | 0.3 | 1.5 | 0.3 |
| 20:3ω6 | 0.6 | 0.0 | 0.4 | 0.1 | 0.6 | 0.0 | 0.5 | 0.1 | 0.6 | 0.1 | 0.6 | 0.1 | 0.6 | 0.0 | 0.5 | 0.1 | 0.6 | 0.0 | 0.6 | 0.0 | 0.6 | 0.0 |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 20:0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.3 | 0.0 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.3 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:6ω3 | 0.8 | 0.1 | 0.8 | 0.3 | 0.2 | 0.2 | 0.7 | 0.1 | 0.7 | 0.1 | 0.1 | 0.4 | 0.1 | 0.1 | 0.8 | 0.3 | 0.2 | 0.1 | 0.0 | 0.2 | 0.0 | 0.1 |
| 22:5ω3 | 0.5 | 0.4 | 0.3 | 0.5 | 0.7 | 0.2 | 0.7 | 0.7 | 0.2 | 0.1 | 0.8 | 0.6 | 0.8 | 0.1 | 0.2 | 0.4 | 0.8 | 0.2 | 0.6 | 0.3 | 0.5 | 0.1 |
| 22:4ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 2 | 0 | 3 | 2 | 2 | 0 | 2 | 1 | 1 | 1 | 4 | 2 | 2 | 0 | 3 | 2 | 2 | 0 | 3 | 1 | 1 | 0 |
| 23: | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. |
| 0 | 2 | 1 | 1 | 1 | 3 | 0 | 2 | 2 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 0 | 3 | 1 | 2 | 0 | 2 | 1 |
| 24: | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. |
| 0 | 1 | 0 | 2 | 1 | 2 | 0 | 1 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 2 | 0 | 2 | 0 | 1 | 0 |
| ∑SF | 38 | 1. | 45 | 1. | 39 | 2. | 38 | 1. | 42 | 3. | 39 | 1. | 36 | 2. | 42 | 1. | 37 | 1. | 39 | 1. | 39 | 2. |
| A | .5 | 1 | .0 | 8 | .3 | 4 | .4 | 2 | .1 | 9 | .4 | 2 | .7 | 0 | .6 | 5 | .5 | 8 | .1 | 1 | .8 | 1 |
| ∑M | 30 | 1. | 30 | 2. | 29 | 1. | 31 | 0. | 28 | 1. | 28 | 1. | 28 | 0. | 31 | 1. | 29 | 1. | 29 | 1. | 28 | 0. |
| UFA | .5 | 5 | .4 | 0 | .9 | 0 | .5 | 3 | .5 | 5 | .5 | 9 | .3 | 6 | .0 | 5 | .5 | 1 | .2 | 0 | .6 | 6 |
| ∑PU | 31 | 2. | 24 | 3. | 30 | 2. | 30 | 1. | 29 | 5. | 32 | 2. | 35 | 2. | 26 | 2. | 33 | 2. | 31 | 1. | 31 | 2. |
| FA | .0 | 3 | .6 | 1 | .8 | 5 | .1 | 5 | .4 | 2 | .0 | 0 | .0 | 5 | .4 | 3 | .0 | 3 | .7 | 5 | .6 | 5 |
| ∑n- | 9. | 1. | 8. | 1. | 7. | 0. | 8. | 0. | 6. | 1. | 10 | 1. | 7. | 0. | 8. | 1. | 7. | 0. | 10 | 0. | 7. | 0. |
| 3 | 2 | 0 | 6 | 8 | 2 | 6 | 3 | 2 | 4 | 0 | .9 | 7 | 7 | 5 | 4 | 3 | 5 | 4 | .3 | 9 | 1 | 5 |
| ∑n- | 21 | 1. | 15 | 1. | 22 | 1. | 21 | 1. | 22 | 4. | 20 | 0. | 26 | 2. | 17 | 1. | 24 | 1. | 21 | 1. | 23 | 2. |
| 6 | .5 | 7 | .6 | 6 | .8 | 9 | .7 | 3 | .5 | 2 | .8 | 8 | .5 | 0 | .7 | 4 | .7 | 8 | .1 | 1 | .9 | 0 |
| ∑Ot | 7. | 0. | 6. | 0. | 8. | 1. | 7. | 0. | 5. | 0. | 7. | 0. | 7. | 0. | 6. | 0. | 8. | 1. | 7. | 0. | 6. | 0. |
| her | 7 | 5 | 5 | 9 | 7 | 3 | 4 | 1 | 3 | 3 | 8 | 7 | 5 | 4 | 7 | 6 | 9 | 3 | 8 | 4 | 6 | 3 |
| FA | | | | | | | | | | | | | | | | | | | | | | |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.3** Sire breed and sex interactions with plane of nutrition on kidney tissue fatty acid composition [1]

| (% total) | **Black Suffolk** | | **Dorset** | | | | **Merino** | | | | **White Suffolk** | | | | **Ewe** | | | | **Wether** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **High (*n* 6)** | | **High (*n* 6)** | | **Low (*n* 8)** | | **High (*n* 2)** | | **Low (*n* 4)** | | **High (*n* 6)** | | **Low (*n* 8)** | | **High (*n* 9)** | | **Low (*n8* )** | | **High (*n* 11)** | | **Low (*n* 12)** | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 0.0 | 0.0 | 0.1 | 0.1 | 1 | 0.1 | 0.5 | 0.4 | 4 | 0.2 | 1 | 1 | 3 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 2 | 1 |
| 15:0 | 0.4 | 0.1 | 3 | 0 | 2 | 1 | 2 | 2 | 3 | 1 | 3 | 0 | 3 | 1 | 3 | 0 | 2 | 1 | 4 | 1 | 3 | 1 |
| 16:1ω9c | 0.2 | 0.0 | 2 | 0.0 | 2 | 0.1 | 3 | 0.3 | 2 | 0.1 | 2 | 0.0 | 1 | 0 | 2 | 0.0 | 1 | 0 | 2 | 0.1 | 2 | 0.0 |
| 16:1ω7c | 0.2 | 0.1 | 3 | 0 | 4 | 1 | 1 | 0.6 | 2 | 1 | 3 | 0.0 | 2 | 1 | 3 | 0.0 | 3 | 1 | 4 | 0.1 | 3 | 0.1 |
| 16:0 | 17.6 | 0.7 | 17.6 | 0.4 | 17.6 | 0.8 | 27.1 | 10.2 | 21.1 | 0.8 | 17.9 | 0.4 | 19.7 | 0.7 | 17.7 | 0.5 | 19.0 | 0.6 | 19.4 | 1.8 | 19.3 | 0.8 |
| 17:0 | 1.3 | 0.1 | 1 | 0 | 4 | 1 | 8 | 3 | 6 | 2 | 1 | 0 | 7 | 1 | 2 | 0 | 6 | 1 | 1 | 1 | 6 | 1 |
| 18:2ω6 | 8.9 | 0.9 | 8.3 | 0.6 | 10.1 | 0.5 | 6.5 | 1.4 | 11.2 | 0.2 | 9.1 | 0.4 | 9.3 | 0.3 | 8.3 | 0.3 | 10.1 | 0.4 | 8.7 | 0.7 | 10.3 | 0.3 |
| 18:3ω3 | 2.3 | 0.3 | 2.4 | 0.2 | 1.4 | 0.2 | 17.2 | 12.6 | 1.8 | 0.4 | 2.7 | 0.2 | 2.2 | 1 | 2.1 | 0.1 | 1.5 | 0.2 | 5.4 | 2.4 | 1.4 | 0.2 |
| 18:1ω9 | 17.4 | 2.6 | 16.0 | 0.8 | 15.0 | 1.3 | 13.9 | 10.2 | 16.7 | 0.7 | 15.3 | 0.7 | 14.7 | 0.6 | 15.1 | 0.5 | 15.1 | 0.5 | 16.7 | 2.0 | 15.3 | 0.9 |
| 18:1ω7c | 1.1 | 0.2 | 1.3 | 0.0 | 1.7 | 0.1 | 2.1 | 1.1 | 1.5 | 0.1 | 1.3 | 0.1 | 1.7 | 0.1 | 1.3 | 0.0 | 1.6 | 0.0 | 1.3 | 0.2 | 1.7 | 0.1 |
| 18:1ω7t | 2.0 | 0.6 | 1.7 | 0.2 | 0.7 | 0.1 | 1.3 | 1.3 | 1.1 | 0.1 | 1.4 | 0.3 | 1.1 | 0.1 | 1.4 | 0.3 | 1.0 | 0.1 | 1.8 | 0.4 | 0.9 | 0.1 |
| 18:0 | 21.6 | 0.7 | 22.3 | 1.0 | 19.3 | 1.3 | 12.8 | 10.2 | 21.1 | 2.3 | 20.0 | 0.5 | 21.2 | 1.6 | 20.6 | 0.5 | 20.9 | 1.3 | 20.4 | 1.9 | 20.1 | 1.3 |
| 20:4ω6 | 7.5 | 1.8 | 6 | 0.7 | 12.1 | 1.5 | 1.4 | 1.4 | 7 | 1.6 | 8.8 | 0.7 | 10.4 | 1.4 | 9.2 | 0.7 | 10.7 | 1.3 | 5.8 | 1.0 | 10.4 | 1.3 |
| 20:5ω3 | 6.3 | 1.3 | 7.0 | 0.9 | 4.0 | 0.8 | 1.6 | 1.6 | 2.5 | 0.9 | 7.2 | 0.5 | 3.4 | 0.6 | 7.3 | 0.3 | 3.4 | 0.7 | 5.6 | 1.0 | 3.5 | 0.6 |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20:3ω6 | 0.5 | 0.1 | 0.6 | 0.1 | 0.8 | 0.1 | 0.2 | 0.2 | 0.7 | 0.1 | 0.6 | 0.1 | 0.7 | 0.1 | 0.6 | 0.1 | 0.7 | 0.1 | 0.5 | 0.1 | 0.7 | 0.1 |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 20:0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 | 0.0 | 0.0 | 0.2 | 0.1 | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | 0.2 | 0.0 | 0.0 | 0.7 | 0.4 | 0.0 | 0.0 | 0.7 | 0.3 | 0.0 | 0.0 | 0.6 | 0.2 | 0.0 | 0.0 | 0.6 | 0.2 |
| 22:6ω3 | 2.0 | 0.0 | 2.0 | 0.0 | 3.8 | 0.3 | 1.3 | 1.3 | 2.3 | 0.0 | 2.5 | 0.2 | 2.8 | 0.4 | 2.6 | 0.2 | 2.4 | 0.4 | 2.1 | 0.4 | 3.0 | 0.3 |
| 22:5ω3 | 3.0 | 0.6 | 3.3 | 0.3 | 8.2 | 2.3 | 7.7 | 7.7 | 2.3 | 3.3 | 4.1 | 1.5 | 5.4 | 4.1 | 4.2 | 2.8 | 8.3 | 3.3 | 8.4 | 4.1 | 5.2 | 2.3 |
| 22:0 | 1.0 | 0.3 | 1.0 | 0.2 | 1.0 | 0.4 | 1.1 | 1.1 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 1.0 | 0.0 |
| 23:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24:0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.2 | 0.3 | 0.3 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 | 0.2 | 0.0 | 0.2 | 0.1 | 0.4 | 0.1 |
| ∑SFA | 44.8 | 0.5 | 44.5 | 0.5 | 42.4 | 2.0 | 46.7 | 3.6 | 47.3 | 2.9 | 43.1 | 0.5 | 45.9 | 2.5 | 43.6 | 0.4 | 44.7 | 2.0 | 44.8 | 0.7 | 44.8 | 2.0 |
| ∑MUFA | 24.2 | 2.7 | 23.0 | 0.8 | 21.5 | 1.4 | 22.8 | 8.4 | 22.0 | 0.8 | 22.1 | 1.5 | 21.0 | 0.5 | 22.0 | 0.5 | 21.0 | 0.6 | 23.1 | 1.9 | 22.0 | 0.5 |
| ∑PUFA | 31.7 | 3.0 | 32.0 | 1.8 | 36.5 | 2.4 | 30.4 | 4.8 | 30.8 | 2.3 | 34.1 | 1.5 | 32.5 | 2.8 | 33.5 | 0.7 | 33.5 | 2.5 | 31.9 | 1.9 | 33.5 | 2.5 |
| ∑n-3 | 13.9 | 2.1 | 15.0 | 0.9 | 12.1 | 1.5 | 21.9 | 7.8 | 9.0 | 1.5 | 15.9 | 0.7 | 10.0 | 1.4 | 15.6 | 0.4 | 10.9 | 1.2 | 16.2 | 1.8 | 10.5 | 1.1 |
| ∑n-6 | 17.8 | 1.3 | 16.5 | 1.1 | 23.5 | 1.5 | 8.3 | 3.1 | 20.4 | 1.5 | 18.6 | 1.0 | 21.9 | 1.4 | 18.2 | 0.8 | 22.4 | 1.2 | 15.1 | 1.4 | 22.2 | 1.3 |
| ∑OtherFA | 5.1 | 0.4 | 4.8 | 0.3 | 8.5 | 0.6 | 6.1 | 1.8 | 8.3 | 3.9 | 4.2 | 0.3 | 5.3 | 0.5 | 4.2 | 0.2 | 9.2 | 2.4 | 4.9 | 0.5 | 5.1 | 0.5 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.4** Sire breed and sex interactions with plane of nutrition on liver tissue fatty acid composition [1]

| (% total) | Black Suffolk | | Dorset | | | | Merino | | | | White Suffolk | | | | Ewe | | | | Wether | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High (*n* 6) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 2) | | Low (*n* 4) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 9) | | Low (*n* 8) | | High (*n* 11) | | Low (*n* 12) | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 0.50 | 0.01 | 0.21 | 0.05 | 0.51 | 0.06 | 0.44 | 0.04 | 0.66 | 0.13 | 0.31 | 0.04 | 0.41 | 0.04 | 0.21 | 0.04 | 0.41 | 0.04 | 0.41 | 0.05 | 0.62 | 0.02 |
| 15:0 | 0.40 | 0.00 | 0.31 | 0.06 | 0.60 | 0.05 | 0.40 | 0.00 | 0.81 | 0.10 | 0.50 | 0.00 | 0.51 | 0.06 | 0.31 | 0.06 | 0.51 | 0.06 | 0.41 | 0.06 | 0.61 | 0.05 |
| 16:1ω9c | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |
| 16:1ω7c | 0.70 | 0.01 | 0.51 | 0.06 | 0.61 | 0.07 | 0.52 | 0.02 | 0.63 | 0.08 | 0.51 | 0.01 | 0.61 | 0.07 | 0.61 | 0.06 | 0.61 | 0.06 | 0.51 | 0.01 | 0.61 | 0.06 |
| 16:0 | 16.06 | 0.29 | 16.92 | 0.23 | 21.06 | 0.66 | 18.13 | 1.33 | 26.42 | 2.33 | 16.97 | 0.27 | 22.08 | 0.70 | 17.04 | 0.14 | 22.08 | 0.70 | 16.97 | 0.27 | 22.08 | 1.33 |
| 17:0 | 1.30 | 0.06 | 1.02 | 0.01 | 1.08 | 0.01 | 1.00 | 0.00 | 1.08 | 0.02 | 1.01 | 0.00 | 1.09 | 0.01 | 1.01 | 0.00 | 1.08 | 0.01 | 1.01 | 0.00 | 1.08 | 0.01 |
| 18:2ω6 | 5.61 | 0.26 | 5.11 | 1.46 | 6.91 | 0.45 | 7.05 | 0.05 | 9.66 | 0.66 | 5.90 | 0.97 | 6.75 | 0.45 | 5.31 | 0.70 | 7.45 | 0.45 | 6.61 | 0.97 | 8.45 | 0.26 |
| 18:3ω6 | 3.02 | 0.22 | 2.32 | 0.44 | 4.24 | 0.44 | 3.44 | 0.74 | 8.48 | 1.84 | 2.64 | 0.44 | 4.24 | 0.44 | 2.32 | 0.44 | 4.24 | 0.44 | 2.64 | 0.44 | 4.24 | 0.22 |
| 18:1ω9 | 22.31 | 1.03 | 20.41 | 2.03 | 23.21 | 1.03 | 21.55 | 0.05 | 25.00 | 0.97 | 18.41 | 1.03 | 24.21 | 1.03 | 21.41 | 1.03 | 24.21 | 1.03 | 20.41 | 1.03 | 23.21 | 0.97 |
| 18:0 | 0.31 | 0.01 | 0.41 | 0.06 | 0.21 | 0.06 | 0.05 | 0.05 | 0.09 | 0.09 | 0.71 | 0.01 | 0.11 | 0.06 | 0.41 | 0.06 | 0.21 | 0.06 | 0.01 | 0.01 | 0.31 | 0.06 |
| 18:1ω7 | 1.30 | 0.06 | 1.02 | 0.01 | 1.08 | 0.01 | 1.00 | 0.00 | 1.08 | 0.02 | 1.01 | 0.00 | 1.09 | 0.01 | 1.01 | 0.00 | 1.08 | 0.01 | 1.01 | 0.00 | 1.08 | 0.01 |
| 18:1ω5 | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |
| 18:1 | 0.70 | 0.01 | 0.51 | 0.06 | 0.61 | 0.07 | 0.52 | 0.02 | 0.63 | 0.08 | 0.51 | 0.01 | 0.61 | 0.07 | 0.61 | 0.06 | 0.61 | 0.06 | 0.51 | 0.01 | 0.61 | 0.06 |
| 19:1 | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |
| 20:1ω11 | 0.70 | 0.01 | 0.51 | 0.06 | 0.61 | 0.07 | 0.52 | 0.02 | 0.63 | 0.08 | 0.51 | 0.01 | 0.61 | 0.07 | 0.61 | 0.06 | 0.61 | 0.06 | 0.51 | 0.01 | 0.61 | 0.06 |
| 20:1ω9 | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |
| 20:1ω7 | 0.70 | 0.01 | 0.51 | 0.06 | 0.61 | 0.07 | 0.52 | 0.02 | 0.63 | 0.08 | 0.51 | 0.01 | 0.61 | 0.07 | 0.61 | 0.06 | 0.61 | 0.06 | 0.51 | 0.01 | 0.61 | 0.06 |
| 20:1ω5 | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |
| 22:1ω9 | 0.70 | 0.01 | 0.51 | 0.06 | 0.61 | 0.07 | 0.52 | 0.02 | 0.63 | 0.08 | 0.51 | 0.01 | 0.61 | 0.07 | 0.61 | 0.06 | 0.61 | 0.06 | 0.51 | 0.01 | 0.61 | 0.06 |
| 22:1ω11 | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |
| 22:1ω7 | 0.70 | 0.01 | 0.51 | 0.06 | 0.61 | 0.07 | 0.52 | 0.02 | 0.63 | 0.08 | 0.51 | 0.01 | 0.61 | 0.07 | 0.61 | 0.06 | 0.61 | 0.06 | 0.51 | 0.01 | 0.61 | 0.06 |
| 24:1ω11 | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |
| 24:1ω9 | 0.70 | 0.01 | 0.51 | 0.06 | 0.61 | 0.07 | 0.52 | 0.02 | 0.63 | 0.08 | 0.51 | 0.01 | 0.61 | 0.07 | 0.61 | 0.06 | 0.61 | 0.06 | 0.51 | 0.01 | 0.61 | 0.06 |
| 24:1ω7 | 0.40 | 0.00 | 0.31 | 0.05 | 0.50 | 0.06 | 0.42 | 0.02 | 0.71 | 0.10 | 0.30 | 0.00 | 0.51 | 0.06 | 0.31 | 0.05 | 0.51 | 0.06 | 0.40 | 0.00 | 0.50 | 0.05 |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1ω7c | 4 | 0 | 0 | 2 | 2 | 0 | 2 | 1 | 2 | 1 | .4 | 1 | 3 | 1 | 2 | 2 | 3 | 1 | 3 | 1 | 2 | 0 |
| 18:1ω7t | 2.5 | 0.7 | 2.4 | 0.5 | 1.9 | 0.1 | 2.6 | 1.2 | 2.1 | 0.2 | 1.9 | 0.6 | 2.2 | 0.1 | 2.4 | 0.5 | 2.0 | 0.1 | 2.2 | 0.4 | 2.1 | 0.1 |
| 18:0 | 24.5 | 0.9 | 27.3 | 3.8 | 20.8 | 2.1 | 20.9 | 0.4 | 17.5 | 2.6 | 24.5 | 1.2 | 21.7 | 1.9 | 26.0 | 2.6 | 20.3 | 1.5 | 24.1 | 0.9 | 20.7 | 1.8 |
| 20:4ω6 | 3.6 | 0.2 | 4.0 | 1.2 | 4.8 | 0.7 | 5.7 | 3.4 | 3.1 | 1.4 | 4.8 | 1.2 | 6.9 | 0.3 | 4.0 | 0.9 | 4.3 | 0.7 | 5.7 | 0.7 | 5.7 | 0.7 |
| 20:5ω3 | 3.9 | 0.1 | 3.0 | 0.9 | 1.9 | 0.4 | 5.1 | 2.7 | 1.0 | 0.6 | 4.6 | 0.5 | 1.5 | 0.4 | 3.0 | 0.6 | 1.8 | 0.4 | 4.6 | 0.5 | 1.5 | 0.4 |
| 20:3ω6 | 0.5 | 0.1 | 0.7 | 0.9 | 0.9 | 0.4 | 0.0 | 0.0 | 0.0 | 0.6 | 0.5 | 0.5 | 0.5 | 0.4 | 0.7 | 0.6 | 0.3 | 0.4 | 0.0 | 0.5 | 0.0 | 0.0 |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:0 | 1.0 | 0.0 | 1.0 | 0.0 | 1.1 | 1.0 | 2.1 | 1.0 | 1.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 1.0 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 | 2.1 | 0.0 | 0.0 | 0.0 | 7.6 | 0.0 | 0.0 | 1.1 | 0.0 | 0.0 | 0.0 | 6.4 | 0.0 | 0.0 |
| 22:6ω3 | 3.2 | 0.3 | 3.8 | 0.2 | 2.8 | 0.8 | 8.5 | 5.5 | 1.5 | 0.5 | 1.5 | 0.4 | 2.6 | 0.5 | 2.5 | 5.7 | 4.4 | 1.6 | 4.4 | 1.6 | 3.4 | 0.3 |
| 22:5ω3 | 4.0 | 0.2 | 3.0 | 0.8 | 2.0 | 0.4 | 3.0 | 0.6 | 1.0 | 0.4 | 4.0 | 0.2 | 2.0 | 0.3 | 3.0 | 0.6 | 2.0 | 0.4 | 4.0 | 0.2 | 2.0 | 0.3 |
| 22:0 | 2.5 | 0.5 | 7.8 | 4.3 | 7.4 | 0.5 | 5.6 | 6.4 | 4.6 | 6.7 | 7.4 | 4.3 | 3.4 | 4.5 | 8.5 | 2.4 | 4.4 | 3.4 | 4.4 | 3.4 | 4.4 | 3.4 |
| 23:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24:0 | 2.1 | 0.1 | 2.1 | 1.1 | 2.1 | 1.1 | 2.2 | 1.2 | 1.1 | 1.1 | 3.1 | 1.1 | 1.1 | 1.1 | 2.1 | 1.1 | 1.1 | 1.1 | 3.1 | 1.1 | 2.1 | 1.1 |
| ∑SFA | 44.7 | 0.8 | 47.5 | 3.6 | 45.9 | 2.4 | 43.7 | 0.2 | 48.3 | 4.2 | 45.0 | 0.4 | 47.8 | 2.4 | 46.3 | 2.4 | 46.1 | 1.9 | 44.8 | 0.6 | 47.2 | 2.1 |
| ∑MUFA | 30.4 | 1.2 | 27.5 | 1.4 | 30.1 | 1.1 | 29.0 | 6.7 | 32.7 | 1.2 | 26.1 | 1.7 | 31.0 | 1.0 | 28.4 | 1.5 | 31.5 | 1.1 | 27.5 | 3.8 | 30.6 | 0.8 |
| ∑PUFA | 24.9 | 1.9 | 25.0 | 4.2 | 24.0 | 3.2 | 27.3 | 7.4 | 19.1 | 4.0 | 28.9 | 1.6 | 21.2 | 2.7 | 24.9 | 2.6 | 22.4 | 2.1 | 27.7 | 1.6 | 21.2 | 2.9 |
| ∑n-3 | 14.2 | 0.8 | 14.2 | 2.6 | 11.4 | 1.4 | 13.3 | 3.0 | 7.8 | 2.5 | 17.4 | 0.7 | 9.5 | 1.7 | 14.3 | 1.6 | 10.2 | 1.7 | 16.1 | 0.9 | 9.8 | 1.5 |
| ∑n-6 | 9.5 | 0.7 | 10.2 | 2.3 | 12.3 | 1.3 | 13.4 | 4.1 | 11.1 | 1.4 | 11.1 | 1.1 | 11.1 | 1.1 | 10.1 | 1.1 | 11.1 | 1.1 | 11.1 | 1.1 | 11.1 | 0.9 |
| ∑OtherFA | 4.6 | 0.1 | 4.1 | 0.3 | 4.1 | 0.2 | 4.7 | 0.4 | 2.5 | 0.5 | 5.3 | 0.6 | 3.6 | 0.3 | 4.3 | 0.6 | 3.3 | 0.3 | 5.5 | 0.1 | 4.1 | 0.2 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.5** Sire breed and sex interactions with plane of nutrition on *Longissimus dorsi* muscle tissue fatty acid composition [1]

| (% total) | Black Suffolk | | Dorset | | | | Merino | | | | White Suffolk | | | | Ewe | | | | Wether | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High (*n* 6) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 2) | | Low (*n* 4) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 9) | | Low (*n* 8) | | High (*n* 11) | | Low (*n* 12) | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 2.2 | 0.2 | 2.1 | 0.2 | 3.1 | 0.3 | 1.4 | 0.5 | 4.5 | 0.5 | 1.9 | 0.4 | 5.3 | 0.3 | 1.3 | 0.3 | 4.4 | 0.4 | 0.1 | 0.1 | 4.2 | 0.2 |
| 15:0 | 0.5 | 0.0 | 0.0 | 0.0 | 4.0 | 1.0 | 5.0 | 0.0 | 5.1 | 1.0 | 6.1 | 1.0 | 3.0 | 0.0 | 5.1 | 1.0 | 3.0 | 0.0 | 5.0 | 0.0 | 4.0 | 0.0 |
| 16:1ω9c | 0.3 | 0.0 | 0.3 | 0.0 | 2.0 | 0.0 | 3.0 | 0.0 | 3.0 | 0.0 | 0.3 | 0.0 | 2.0 | 0.0 | 0.3 | 0.0 | 2.0 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 |
| 16:1ω7c | 1.3 | 0.1 | 1.2 | 0.0 | 3.1 | 1.0 | 1.0 | 0.1 | 3.1 | 1.0 | 1.2 | 0.1 | 9.2 | 0.0 | 1.3 | 0.1 | 2.1 | 1.0 | 1.1 | 0.0 | 1.1 | 0.2 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16:0 | 22.8 | 1.0 | 22.6 | 0.5 | 21.7 | 0.5 | 20.3 | 1.0 | 24.1 | 0.8 | 22.8 | 0.6 | 22.1 | 0.7 | 23.3 | 0.6 | 22.3 | 0.6 | 21.8 | 0.4 | 22.4 | 0.5 |
| 17:0 | 1.6 | 0.1 | 2 | 1 | 4 | 1 | 4 | 0 | 5 | 1 | 2 | 0 | 4 | 0 | 3 | 1 | 4 | 1 | 4 | 1 | 4 | 0 |
| 18:2ω6 | 3.7 | 0.2 | 6 | 7 | 0 | 5 | 3 | 2 | 8 | 7 | 0 | 2 | 9 | 6 | 3 | 3 | 7 | 7 | 9 | 4 | 4 | 3 |
| 18:3ω3 | 2.1 | 0.1 | 0 | 1 | 6 | 1 | 7 | 1 | 0 | 2 | 2 | 1 | 8 | 1 | 1 | 1 | 6 | 1 | 2 | 1 | 8 | 1 |
| 18:1ω9 | 35.3 | 2.4 | 36.6 | 1.0 | 36.7 | 0.9 | 32.3 | 1.0 | 34.3 | 2.2 | 34.9 | 0.7 | 37.6 | 1.4 | 34.5 | 1.5 | 36.2 | 1.3 | 35.8 | 0.9 | 36.9 | 0.9 |
| 18:1ω7c | 1.7 | 0.1 | 3 | 0 | 4 | 1 | 2 | 0 | 5 | 1 | 1.5 | 0 | 5 | 1 | 5 | 1 | 4 | 1 | 4 | 1 | 5 | 0 |
| 18:1ω7t | 3.3 | 0.2 | 2.7 | 1 | 5 | 1 | 7 | 3 | 6 | 1 | 3.1 | 1 | 7 | 2 | 2 | 1 | 4 | 2 | 3.0 | 1 | 2.7 | 1 |
| 18:0 | 19.2 | 0.6 | 20.9 | 0.8 | 20.8 | 1.0 | 22.5 | 0.3 | 19.4 | 0.9 | 19.9 | 1.6 | 18.2 | 0.4 | 20.1 | 1.0 | 19.1 | 1.6 | 20.3 | 0.7 | 19.5 | 0.5 |
| 20:4ω6 | 4 | 1 | 5 | 1 | 8 | 2 | 9 | 1 | 7 | 1 | 3 | 1 | 0 | 2 | 4 | 1 | 9 | 2 | 5 | 1 | 8 | 1 |
| 20:5ω3 | 5 | 2 | 5 | 1 | 4 | 1 | 7 | 4 | 3 | 1 | 4 | 2 | 5 | 1 | 5 | 1 | 5 | 1 | 5 | 1 | 4 | 1 |
| 20:3ω6 | 0 | 0 | 1 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 0 |
| 20:4ω3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 20:2ω6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20:0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| 22:5ω6 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 |
| 22:6ω3 | 0 | 0 | 1 | 0 | 2 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 0 |
| 22:5ω3 | 1 | 1 | 2 | 1 | 4 | 1 | 7 | 1 | 2 | 1 | 1 | 1 | 5 | 1 | 1 | 1 | 5 | 1 | 3 | 1 | 4 | 1 |
| 22:0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23:0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24:0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ∑SFA | 47.1 | 1.7 | 47.4 | 1.4 | 46.5 | 1.5 | 47.0 | 0.6 | 48.2 | 0.8 | 47.2 | 2.3 | 44.7 | 0.4 | 48.1 | 1.7 | 46.0 | 1.0 | 46.0 | 0.9 | 45.9 | 0.9 |
| ∑MUFA | 45.2 | 2.6 | 45.1 | 1.0 | 45.1 | 1.1 | 41.0 | 0.3 | 43.1 | 1.3 | 46.1 | 1.9 | 46.1 | 1.3 | 45.1 | 1.7 | 44.1 | 1.3 | 45.9 | 0.9 | 45.9 | 0.9 |
| ∑PUFA | 7.6 | 0.5 | 7.1 | 1.2 | 8.1 | 1.1 | 11.0 | 0.5 | 8.8 | 0.8 | 6.5 | 0.7 | 9.1 | 1.2 | 6.5 | 0.5 | 9.2 | 1.4 | 7.9 | 0.8 | 8.5 | 0.6 |
| ∑n-3 | 2.8 | 0.2 | 2.6 | 0.3 | 7.4 | 4.3 | 4.6 | 0.6 | 2.6 | 0.3 | 2.8 | 0.4 | 3.0 | 0.4 | 2.0 | 0.4 | 2.4 | 0.4 | 1.3 | 7.2 | 2.2 | |
| ∑n-6 | 4.8 | 0.2 | 4.5 | 0.9 | 2.7 | 5.0 | 6.5 | 0.9 | 6.2 | 0.5 | 3.7 | 0.3 | 6.1 | 1.9 | 4.5 | 0.3 | 6.8 | 1.0 | 6.5 | 6.4 | 6.3 | 0.4 |
| ∑Other FA | 4.8 | 0.3 | 4.2 | 0.1 | 2.2 | 2.8 | 8.0 | 0.1 | 4.0 | 0.1 | 6.3 | 1.5 | 4.3 | 0.4 | 6.0 | 1.0 | 4.3 | 0.3 | 5.1 | 0.3 | 4.1 | 0.2 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.6** Sire breed and sex interactions with plane of nutrition on subcutaneous adipose tissue fatty acid content [1]

| (mg/100g) | Black Suffolk | | Dorset | | | | Merino | | | | White Suffolk | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High (*n* 6) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 2) | | Low (*n* 4) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 9) | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| % lipid | 90.5 | 19.3 | 92.6 | 12.7 | 73.1 | 4.3 | 69.9 | 2.7 | 61.3 | 5.9 | 59.9 | 6.7 | 66.8 | 6.2 | 80.7 | 16.0 |
| 14:0 | 1277.7 | 508.8 | 2275.9 | 313.5 | 988.7 | 298.8 | 1547.7 | 389.7 | 903.6 | 219.0 | 1481.8 | 295.8 | 1303.3 | 370.7 | 1805.5 | 366.8 |
| 15:0 | 295.8 | 111.2 | 561.4 | 83.8 | 297.4 | 91.2 | 333.6 | 7.5 | 210.9 | 90.2 | 329.4 | 56.4 | 306.4 | 87.3 | 423.6 | 88.1 |
| 16:1ω9c | 179.0 | 48.0 | 292.4 | 51.2 | 118.5 | 35.0 | 223.7 | 0.3 | 105.6 | 25.5 | 187.4 | 30.3 | 150.1 | 36.8 | 234.3 | 48.4 |
| 16:1ω7c | 585.3 | 185.6 | 1053.4 | 116.2 | 281.9 | 106.5 | 449.9 | 25.3 | 187.0 | 115.9 | 791.3 | 149.0 | 349.2 | 76.4 | 832.8 | 159.0 |
| 16:0 | 10675.1 | 2850.6 | 19572.1 | 2192.4 | 10199.9 | 2700.2 | 9951.6 | 545.2 | 7635.2 | 2230.4 | 12849.5 | 2403.6 | 9840.8 | 1938.5 | 14825.8 | 2775.2 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17:0 | 1061.3 | 280.9 | 1617.1 | 206.6 | 764.2 | 220.5 | 815.4 | 114.0 | 641.0 | 217.2 | 924.9 | 162.0 | 742.3 | 160.9 | 1237.9 | 240.5 | | |
| 18:2ω6 | 714.8 | 194.8 | 1276.2 | 104.7 | 559.2 | 140.4 | 830.6 | 170.1 | 667.8 | 220.7 | 883.6 | 141.0 | 584.3 | 118.7 | 982.0 | 158.9 | | |
| 18:3ω3 | 657.2 | 190.7 | 1302.3 | 140.3 | 466.2 | 126.7 | 685.4 | 71.7 | 499.7 | 141.3 | 918.9 | 174.9 | 494.5 | 109.2 | 975.2 | 174.7 | | |
| 18:1ω9 | 14632.1 | 4129.7 | 28207.4 | 2936.5 | 12719.0 | 3056.0 | 12694.2 | 1676.6 | 8214.1 | 1578.4 | 19244.6 | 3628.7 | 13293.0 | 2312.1 | 22044.5 | 4063.2 | | |
| 18:1ω7c | 667.2 | 184.3 | 1139.3 | 128.3 | 425.7 | 102.9 | 537.6 | 40.4 | 331.2 | 71.3 | 766.2 | 128.0 | 475.4 | 91.4 | 913.8 | 165.2 | | |
| 18:1ω7t | 1774.4 | 451.4 | 3184.7 | 379.1 | 1161.6 | 358.3 | 2110.5 | 198.4 | 854.9 | 169.5 | 1929.3 | 258.4 | 1680.0 | 532.3 | 2332.1 | 402.1 | | |
| 18:0 | 9072.1 | 2430.8 | 20014.8 | 3236.7 | 11000.1 | 3427.9 | 13336.6 | 2594.9 | 7884.7 | 2608.9 | 10922.6 | 1727.7 | 10109.3 | 2571.5 | 13816.8 | 3239.2 | | |
| 20:4ω6 | 8.5 | 8.5 | 0.0 | 0.0 | 16.3 | 5.3 | 0.0 | 0.0 | 9.6 | 9.6 | 11.8 | 8.3 | 13.9 | 5.7 | 2.4 | 2.4 | | |
| 20:5ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 36.5 | 20.2 | 0.0 | 0.0 | 3.5 | 3.5 | 31.5 | 28.3 | 33.3 | 17.7 | 1.9 | 1.9 | | |
| 20:3ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 15.6 | 9.5 | 0.0 | 0.0 | 3.7 | 3.7 | 0.0 | 0.0 | 12.5 | 7.1 | 0.0 | 0.0 | | |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 1.4 | 0.0 | 0.0 | 1.2 | 1.2 | 6.9 | 6.9 | 5.3 | 4.1 | 0.0 | 0.0 | | |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 62.8 | 36.9 | 0.0 | 0.0 | 7.3 | 7.3 | 0.0 | 0.0 | 67.1 | 40.6 | 0.0 | 0.0 | | |
| 20:0 | 0.0 | 0.0 | 15.8 | 15.8 | 74.3 | 29.9 | 2.3 | 2.3 | 58.4 | 26.9 | 25.6 | 11.5 | 58.7 | 17.9 | 5.8 | 5.8 | | |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 39.8 | 16.9 | 0.0 | 0.0 | 87.9 | 67.2 | 11.8 | 11.8 | 31.0 | 16.8 | 0.0 | 0.0 | | |
| 22:6ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 7.8 | 4.2 | 0.0 | 0.0 | 0.0 | 0.0 | 73.1 | 70.9 | 10.4 | 9.2 | 1.2 | 1.2 | | |
| 22:5ω3 | 0.0 | 0.0 | 17.7 | 17.7 | 25.5 | 9.9 | 0.0 | 0.0 | 12.6 | 2.9 | 14.9 | 14.9 | 27.0 | 13.7 | 0.0 | 0.0 | | |
| 22:0 | 0.0 | 0.0 | 3.3 | 3.3 | 9.4 | 4.8 | 0.0 | 0.0 | 6.1 | 3.6 | 0.0 | 0.0 | 6.3 | 3.6 | 0.0 | 0.0 | | |
| 23:0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 1.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | |
| 24:0 | 0.0 | 0.0 | 4.2 | 4.2 | 0.9 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.9 | 0.9 | 0.0 | 0.0 | 2.8 | 2.8 | | |
| SUM | 41600.4 | 11133.2 | 80538.2 | 9415.0 | 39275.3 | 10346.3 | 43519.1 | 4589.5 | 28326.0 | 7614.0 | 51405.9 | 8837.6 | 39594.1 | 8258.7 | 60438.4 | 11489. | | |
| ∑SFA | 22663.4 | 5995.9 | 44770.1 | 6063.5 | 23830.6 | 6839.3 | 26364.8 | 2841.2 | 17523.0 | 5425.8 | 26899.7 | 4661.5 | 22646.1 | 5162.8 | 32585.1 | 6678.9 | | |
| ∑MUFA | 19756.8 | 5413.5 | 36739.3 | 3751.4 | 15861.1 | 3847.6 | 17140.1 | 1511.9 | 10568.1 | 2164.5 | 24959.3 | 4505.7 | 17128.3 | 3171.0 | 28686.7 | 5189.4 | | |
| ∑PUFA | 1489.1 | 420.0 | 2843.7 | 277.4 | 1357.3 | 378.8 | 1627.5 | 233.2 | 1384.5 | 377.0 | 2133.3 | 290.3 | 1407.0 | 358.5 | 2144.6 | 360.5 | | |
| ∑n-3 | 657.2 | 190.7 | 1329.9 | 141.7 | 545.9 | 159.5 | 685.4 | 71.7 | 516.9 | 145.5 | 1068.3 | 151.5 | 571.7 | 148.4 | 983.4 | 173.2 | | |
| ∑n-6 | 723.3 | 190.9 | 1277.2 | 104.7 | 701.1 | 188.7 | 830.6 | 170.1 | 776.2 | 208.5 | 910.3 | 130.0 | 709.9 | 178.6 | 986.5 | 157.6 | | |
| ∑Other FA | 2308.9 | 587.9 | 3814.9 | 466.1 | 1773.7 | 581.5 | 1613.2 | 3.2 | 1149.6 | 306.8 | 2586.4 | 427.2 | 1587.6 | 395.5 | 2978.0 | 539.9 | | |

[1] % lipid is the percentage lipid in raw tissue; ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.7** Sire breed and sex interactions with plane of nutrition on heart tissue fatty acid content [1]

| | **Black Suffolk** | | **Dorset** | | | | **Merino** | | | | **White Suffolk** | | | | **Ewe** | | | | **Wether** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (mg /100g) | **High (*n* 6)** | | **High (*n* 6)** | | **Low (*n* 8)** | | **High (*n* 2)** | | **Low (*n* 4)** | | **High (*n* 6)** | | **Low (*n* 8)** | | **High (*n* 9)** | | **Low (*n* 8)** | | **High (*n* 11)** | | **Low (*n* 12)** | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| % lipid | 2.5 | 0.2 | 3.3 | 0.4 | 4.4 | 0.2 | 3.1 | 0.4 | 3.1 | 0.2 | 2.5 | 0.1 | 2.5 | 0.2 | 3.1 | 0.3 | 4.2 | 0.2 | 2.5 | 0.1 | 2.7 | 0.2 |
| 14:0 | 12.7 | 3.3 | 25.2 | 11.7 | 12.9 | 4.6 | 8.7 | 1.6 | 26.6 | 13.5 | 4.9 | 1.1 | 9.0 | 5.4 | 19.8 | 8.1 | 11.1 | 5.0 | 8.7 | 2.0 | 16.1 | 5.7 |
| 15:0 | 6.4 | 0.7 | 10.0 | 2.3 | 4.4 | 1.6 | 6.1 | 0.1 | 2.4 | 0.7 | 4.9 | 0.7 | 3.0 | 0.6 | 7.9 | 1.8 | 2.7 | 0.8 | 6.3 | 0.5 | 4.0 | 1.0 |
| 16:1ω9c | 3.3 | 0.5 | 5.9 | 1.9 | 3.2 | 0.7 | 3.9 | 0.1 | 4.3 | 1.8 | 2.8 | 0.2 | 3.1 | 1.0 | 4.9 | 1.4 | 4.4 | 0.9 | 3.3 | 0.3 | 3.3 | 0.7 |
| 16:1ω7c | 6.2 | 0.9 | 10.7 | 3.6 | 2.0 | 0.9 | 6.3 | 0.4 | 2.9 | 0.6 | 4.8 | 0.5 | 1.6 | 0.6 | 8.9 | 2.5 | 1.6 | 0.9 | 5.6 | 0.5 | 2.3 | 0.4 |
| 16:0 | 195.2 | 18.2 | 319.0 | 77.5 | 221.7 | 41.5 | 206.9 | 62.2 | 273.1 | 105.8 | 163.1 | 55.3 | 198.4 | 53.2 | 261.7 | 78.8 | 227.0 | 48.7 | 192.9 | 18.7 | 222.6 | 45.6 |
| 17:0 | 19.1 | 1.6 | 27.3 | 6.9 | 21.7 | 23.3 | 17.1 | 1.1 | 24.2 | 9.6 | 13.8 | 1.7 | 19.3 | 5.4 | 22.5 | 5.1 | 22.1 | 4.9 | 17.5 | 1.2 | 20.7 | 4.2 |
| 18:2ω6 | 232.9 | 22.6 | 210.4 | 92.2 | 227.7 | 23.3 | 247.0 | 74.7 | 215.5 | 51.0 | 187.8 | 30.8 | 233.4 | 35.4 | 210.2 | 25.1 | 258.8 | 29.5 | 217.2 | 17.7 | 210.9 | 23.9 |
| 18:3ω3 | 537.7 | 66.7 | 654.4 | 111.9 | 313.6 | 36.6 | 576.6 | 97.3 | 373.3 | 91.1 | 519.3 | 95.3 | 315.5 | 50.4 | 554.4 | 94.4 | 330.5 | 44.5 | 584.4 | 49.9 | 325.3 | 40.0 |
| 18:1ω9 | 2788.3 | 355.7 | 4317.1 | 1343.5 | 2911.4 | 523.3 | 2866.7 | 636.3 | 3288.3 | 1317.7 | 2044.3 | 282.8 | 2666.1 | 656.5 | 3688.2 | 946.4 | 3020.5 | 692.4 | 2525.5 | 222.5 | 2779.2 | 566.9 |

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18:1ω7c | 28.5 | 1.7 | 34.7 | 8.5 | 30.1 | 4.1 | 28.8 | 3.2 | 28.1 | 9.6 | 22.6 | 2.9 | 26.9 | 5.2 | 32.1 | 5.8 | 31.8 | 4.4 | 25.8 | 1.7 | 26.2 | 4.2 |
| 18:1ω7t | 38.1 | 3.9 | 58.2 | 17.4 | 27.4 | 8.5 | 37.3 | 2.7 | 32.4 | 13.8 | 26.3 | 3.2 | 24.4 | 10.2 | 49.0 | 12.2 | 26.1 | 9.9 | 33.6 | 3.2 | 27.9 | 7.2 |
| 18:0 | 309.4 | 31.9 | 518.9 | 145.6 | 332.0 | 79.6 | 282.4 | 18.0 | 352.1 | 155.0 | 248.8 | 23.9 | 288.0 | 101.9 | 422.1 | 106.2 | 326.4 | 94.2 | 293.5 | 19.7 | 313.0 | 74.9 |
| 20:4ω6 | 69.2 | 9.0 | 58.7 | 10.7 | 72.1 | 8.2 | 52.4 | 7.9 | 49.9 | 11.3 | 49.3 | 8.4 | 71.0 | 11.6 | 53.0 | 7.4 | 83.7 | 10.0 | 62.8 | 6.7 | 56.2 | 6.3 |
| 20:5ω3 | 46.7 | 6.7 | 46.8 | 9.6 | 23.1 | 2.0 | 39.2 | 2.2 | 17.6 | 3.9 | 37.0 | 8.6 | 24.5 | 4.2 | 40.4 | 6.9 | 27.0 | 3.6 | 45.3 | 5.4 | 19.6 | 2.0 |
| 20:3ω6 | 8.3 | 1.1 | 7.0 | 0.8 | 8.7 | 1.1 | 7.3 | 0.6 | 6.4 | 1.8 | 7.7 | 1.5 | 7.7 | 1.4 | 6.9 | 0.9 | 9.4 | 1.2 | 8.2 | 0.7 | 6.8 | 1.0 |
| 20:4ω3 | 0.6 | 0.3 | 0.4 | 0.3 | 0.8 | 0.8 | 0.8 | 0.8 | 1.7 | 0.7 | 0.7 | 0.5 | 0.6 | 0.4 | 0.5 | 0.3 | 0.7 | 0.5 | 0.6 | 0.2 | 0.6 | 0.6 |
| 20:2ω6 | 0.2 | 0.1 | 0.1 | 0.1 | 0.6 | 0.4 | 0.0 | 0.0 | 0.8 | 0.5 | 0.1 | 0.1 | 0.9 | 0.2 | 0.0 | 0.0 | 0.4 | 0.3 | 0.2 | 0.1 | 0.0 | 0.3 |
| 20:0 | 1.7 | 0.5 | 2.0 | 0.5 | 4.6 | 1.0 | 1.2 | 1.2 | 3.9 | 2.2 | 1.3 | 0.4 | 3.0 | 1.0 | 2.4 | 0.4 | 5.9 | 0.9 | 1.9 | 0.3 | 4.0 | 1.0 |
| 22:5ω6 | 0.2 | 0.1 | 0.0 | 0.0 | 0.2 | 0.2 | 0.0 | 0.0 | 0.2 | 0.2 | 0.1 | 0.1 | 0.7 | 0.3 | 0.2 | 0.1 | 0.3 | 0.3 | 0.0 | 0.0 | 0.4 | 0.1 |
| 22:6ω3 | 11.7 | 2.1 | 11.8 | 3.5 | 16.3 | 2.2 | 10.4 | 1.1 | 8.5 | 2.4 | 11.9 | 3.1 | 13.9 | 2.3 | 9.9 | 2.4 | 17.7 | 2.3 | 13.0 | 1.7 | 11.2 | 1.5 |
| 22:5ω3 | 23.5 | 5.8 | 17.9 | 4.6 | 23.7 | 3.0 | 10.0 | 10.0 | 17.2 | 6.0 | 20.8 | 5.3 | 23.5 | 4.6 | 16.6 | 4.5 | 27.9 | 3.8 | 22.1 | 3.5 | 18.6 | 2.8 |
| 22:0 | 2.6 | 0.5 | 4.4 | 2.2 | 3.1 | 0.5 | 2.3 | 1.5 | 2.2 | 1.3 | 4.5 | 1.8 | 2.6 | 0.7 | 2.9 | 1.3 | 3.3 | 0.5 | 4.3 | 1.1 | 2.3 | 0.6 |
| 23:0 | 3.0 | 0.7 | 2.3 | 1.1 | 4.5 | 0.7 | 2.6 | 2.6 | 2.7 | 1.6 | 2.9 | 1.2 | 4.1 | 1.2 | 2.1 | 0.8 | 5.0 | 0.8 | 3.0 | 0.7 | 3.9 | 0.9 |
| 24:0 | 1.7 | 0.4 | 2.4 | 0.9 | 2.7 | 0.4 | 1.0 | 1.0 | 1.8 | 1.0 | 2.4 | 0.6 | 2.2 | 0.7 | 1.6 | 0.5 | 2.9 | 0.4 | 2.5 | 0.5 | 1.9 | 0.5 |
| SUM | 1353.1 | 123.3 | 1876.7 | 427.6 | 1370.2 | 222.3 | 1315.7 | 6.4 | 1439.5 | 526.5 | 1074.7 | 105.4 | 1260.7 | 310.3 | 1598.1 | 318.9 | 1423.1 | 274.9 | 1279.5 | 6 | 1285.0 | 234.6 |
| ∑SFA | 564.4 | 56.0 | 930.0 | 245.4 | 623.4 | 133.6 | 545.4 | 20.4 | 701.4 | 294.4 | 457.3 | 434 | 543.9 | 171.0 | 758.8 | 181.0 | 617.8 | 155.8 | 543.3 | 34.0 | 600.2 | 134.4 |
| ∑MUFA | 445.0 | 41.5 | 636.3 | 169.7 | 451.1 | 74.4 | 447.8 | 6.5 | 454.5 | 177.1 | 337.3 | 437 | 396.2 | 106.7 | 543.3 | 120.9 | 469.1 | 95.6 | 410.5 | 31.5 | 404.3 | 78.2 |
| ∑PUFA | 454.1 | 48.0 | 430.9 | 255.8 | 424.0 | 43.3 | 427.8 | 18.0 | 363.1 | 186.0 | 374.0 | 435 | 423.9 | 767.1 | 403.9 | 395.3 | 475.2 | 551.8 | 434.6 | 21.4 | 368.8 | 41.7 |
| ∑n-3 | 136.6 | 20.4 | 143.4 | 106.0 | 99.0 | 100.0 | 118.5 | 2.8 | 81.9 | 21.3 | 123.5 | 165 | 95.8 | 126.2 | 123.9 | 183.8 | 103.6 | 93.6 | 140.3 | 99.1 | 84.1 | 100.0 |
| ∑n-6 | 312.4 | 29.8 | 279.5 | 228.4 | 314.8 | 32.4 | 307.7 | 16.8 | 279.4 | 163.1 | 246.9 | 303 | 317.1 | 949.4 | 273.0 | 238.5 | 358.1 | 411.1 | 290.7 | 15.7 | 277.7 | 31.0 |
| ∑Other FA | 110.3 | 7.5 | 120.5 | 162.3 | 128.3 | 23.4 | 105.1 | 1.7 | 79.5 | 28.5 | 94.0 | 159 | 102.1 | 221.1 | 106.6 | 129.0 | 138.0 | 250.0 | 109.1 | 8.1 | 88.1 | 14.5 |

[1] % lipid is the percentage lipid in raw tissue; ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.8** Sire breed and sex interactions with plane of nutrition on kidney tissue fatty acid content [1]

| | Black Suffolk | Dorset | | Merino | | White Suffolk | | Ewe | | Wether | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (mg/100 g) | High (*n* 6) | High (*n* 6) | Low (*n* 8) | High (*n* 2) | Low (*n* 4) | High (*n* 6) | Low (*n* 8) | High (*n* 9) | Low (*n* 8) | High (*n* 11) | Low (*n* 12) |

| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % lipid | 3.0 | 0.2 | 3.0 | 0.1 | 3.2 | 0.2 | 3.2 | 0.0 | 3.4 | 0.2 | 3.0 | 0.1 | 3.1 | 0.1 | 2.9 | 0.1 | 3.1 | 0.1 | 1.1 | 1.1 | 3.1 | 0.1 |
| 14:0 | 0.8 | 0.8 | 1.1 | 1.1 | 1.1 | 0.6 | 7.2 | 7.0 | 3.8 | 2.9 | 2.2 | 1.4 | 2.9 | 1.5 | 2.0 | 1.0 | 2.3 | 1.3 | 1.9 | 1.4 | 2.1 | 1.1 |
| 15:0 | 3.3 | 0.7 | 3.7 | 0.9 | 1.5 | 0.6 | 2.8 | 2.8 | 3.1 | 1.7 | 4.1 | 0.5 | 2.5 | 0.9 | 4.5 | 0.6 | 1.9 | 0.7 | 2.9 | 0.6 | 2.4 | 0.7 |
| 16:1 ω9c | 1.8 | 0.6 | 2.2 | 0.8 | 1.1 | 0.4 | 5.9 | 5.9 | 2.6 | 1.5 | 2.5 | 0.5 | 1.3 | 0.5 | 2.9 | 0.5 | 1.5 | 0.5 | 2.3 | 1.0 | 1.5 | 0.6 |
| 16:1 ω7c | 2.5 | 0.8 | 3.7 | 1.4 | 1.8 | 0.4 | 6.8 | 2.8 | 1.9 | 1.0 | 3.4 | 0.6 | 1.5 | 0.4 | 4.0 | 0.6 | 1.4 | 0.4 | 3.2 | 1.0 | 1.8 | 0.4 |
| 16:0 | 153.8 | 42.3 | 183.7 | 42.4 | 105.7 | 26.9 | 186.8 | 102.4 | 165.9 | 67.9 | 217.2 | 29.3 | 129.3 | 32.2 | 230.4 | 28.4 | 130.0 | 32.1 | 140.0 | 26.1 | 125.3 | 28.3 |
| 17:0 | 11.5 | 3.4 | 11.9 | 2.8 | 8.2 | 1.9 | 10.1 | 9.0 | 11.7 | 4.5 | 13.3 | 1.8 | 10.2 | 2.2 | 15.3 | 2.2 | 10.2 | 2.0 | 9.3 | 1.8 | 9.5 | 1.9 |
| 18:2 ω6 | 74.4 | 20.3 | 82.9 | 16.4 | 57.4 | 12.8 | 73.8 | 62.3 | 91.9 | 39.4 | 110.6 | 16.0 | 69.1 | 18.7 | 109.5 | 14.1 | 69.8 | 17.5 | 70.0 | 13.0 | 68.4 | 15.9 |
| 18:3 ω3 | 17.7 | 3.6 | 26.7 | 7.0 | 8.0 | 1.8 | 72.9 | 5.8 | 17.9 | 9.7 | 31.1 | 3.3 | 9.6 | 3.0 | 28.0 | 4.1 | 10.1 | 2.5 | 31.5 | 7.3 | 11.0 | 3.6 |
| 18:1 ω9 | 134.3 | 30.8 | 174.5 | 47.2 | 82.8 | 17.2 | 210.3 | 202.1 | 133.7 | 55.4 | 185.0 | 26.8 | 97.6 | 25.5 | 196.4 | 23.8 | 101.5 | 24.6 | 146.9 | 37.9 | 97.2 | 21.8 |
| 18:1 ω7c | 10.8 | 3.2 | 12.9 | 2.8 | 9.5 | 2.0 | 12.1 | 5.0 | 11.9 | 5.0 | 15.4 | 2.3 | 11.5 | 2.9 | 16.7 | 2.0 | 10.8 | 2.6 | 9.9 | 1.7 | 10.8 | 2.2 |
| 18:1 ω7t | 12.5 | 4.2 | 19.4 | 6.1 | 5.0 | 1.6 | 22.6 | 22.6 | 9.5 | 4.6 | 17.0 | 5.5 | 7.1 | 1.8 | 17.8 | 4.1 | 6.7 | 1.6 | 16.2 | 4.8 | 6.9 | 1.9 |
| 18:0 | 190.0 | 51.6 | 236.5 | 58.3 | 111.6 | 27.1 | 199.4 | 193.7 | 151.3 | 56.9 | 242.5 | 33.8 | 128.8 | 29.3 | 268.4 | 32.2 | 134.0 | 29.7 | 181.5 | 41.6 | 125.4 | 25.0 |
| 20:4 ω6 | 90.0 | 37.9 | 79.6 | 21.7 | 76.1 | 20.5 | 24.4 | 24.4 | 73.7 | 35.1 | 110.1 | 19.9 | 83.5 | 25.7 | 128.2 | 23.2 | 85.0 | 24.4 | 52.1 | 11.4 | 74.3 | 17.8 |
| 20:5 ω3 | 63.8 | 18.9 | 69.4 | 17.5 | 26.2 | 8.1 | 26.9 | 26.9 | 28.3 | 15.2 | 85.1 | 10.0 | 29.0 | 9.6 | 94.8 | 12.3 | 29.7 | 9.7 | 46.4 | 8.1 | 26.5 | 6.9 |
| 20:3 ω6 | 5.7 | 1.7 | 6.4 | 2.0 | 4.7 | 1.3 | 3.4 | 3.4 | 5.5 | 2.5 | 8.2 | 1.6 | 5.1 | 1.5 | 7.9 | 1.3 | 5.6 | 1.6 | 5.1 | 1.4 | 4.6 | 1.1 |
| 20:4 ω3 | 0.8 | 0.6 | 0.6 | 0.6 | 0.4 | 0.2 | 0.0 | 0.0 | 2.8 | 0.8 | 1.1 | 1.1 | 4.2 | 0.2 | 0.5 | 0.5 | 5.2 | 0.3 | 0.0 | 0.0 | 7.3 | 0.3 |
| 20:2 ω6 | 0.1 | 0.1 | 3.2 | 2.5 | 5.2 | 2.0 | 0.0 | 0.0 | 7.4 | 4.2 | 2.1 | 1.8 | 8.2 | 2.1 | 2.1 | 1.8 | 8.2 | 2.1 | 1.1 | 1.1 | 6.2 | 2.0 |
| 20:0 | 1.7 | 1.0 | 2.9 | 0.1 | 1.3 | 0.3 | 7.7 | 7.7 | 6.6 | 1.0 | 3.1 | 0.7 | 4.4 | 0.4 | 2.9 | 0.8 | 5.3 | 0.3 | 1.0 | 0.4 | 2.3 | 1.0 |
| 22:5 ω6 | 0.1 | 0.1 | 3.3 | 3.6 | 6.5 | 2.1 | 0.0 | 0.0 | 7.0 | 1.1 | 1.1 | 0.5 | 8.6 | 1.0 | 1.1 | 0.5 | 8.5 | 1.0 | 1.1 | 1.1 | 6.6 | 1.0 |
| 22:6 ω3 | 22.4 | 8.6 | 33.0 | 11.0 | 24.6 | 7.2 | 22.2 | 22.2 | 19.1 | 9.1 | 30.3 | 3.8 | 22.8 | 7.0 | 35.1 | 6.1 | 25.7 | 7.2 | 22.1 | 6.1 | 21.4 | 5.3 |
| 22:5 ω3 | 32.8 | 12.6 | 38.4 | 12.2 | 17.4 | 4.8 | 28.5 | 28.5 | 19.4 | 8.7 | 41.5 | 6.4 | 19.7 | 6.2 | 46.7 | 8.0 | 20.2 | 5.5 | 28.4 | 7.5 | 17.8 | 4.5 |
| 22:0 | 13.9 | 4.7 | 12.9 | 4.1 | 5.0 | 1.1 | 4.7 | 1.9 | 4.8 | 1.7 | 16.0 | 2.3 | 4.2 | 1.2 | 19.0 | 3.1 | 4.7 | 1.1 | 8.7 | 1.7 | 4.6 | 1.0 |
| 23:0 | 2.3 | 1.5 | 2.3 | 1.5 | 1.8 | 0.5 | 0.7 | 0.7 | 1.9 | 0.5 | 3.8 | 0.9 | 1.5 | 0.4 | 4.1 | 1.1 | 1.6 | 0.5 | 1.4 | 0.6 | 1.7 | 0.4 |
| 24:0 | 9.7 | 3.9 | 8.3 | 4.3 | 5.4 | 1.2 | 4.0 | 2.7 | 4.6 | 1.7 | 11.7 | 2.3 | 3.6 | 0.9 | 14.2 | 3.2 | 4.3 | 1.0 | 5.3 | 1.1 | 4.7 | 0.9 |
| SUM | 856.5 | 247.7 | 1011.7 | 246.5 | 559.8 | 132.5 | 926.1 | 726.2 | 768.3 | 329.3 | 1115.4 | 157.0 | 645.1 | 164.9 | 1250.2 | 162.2 | 665.2 | 165.9 | 796.4 | 154.8 | 614.3 | 136.5 |
| ∑SFA | 394.9 | 111.6 | 469.8 | 111.6 | 246.0 | 59.8 | 425.7 | 312.5 | 356.0 | 146.3 | 523.1 | 71.4 | 293.2 | 69.2 | 577.1 | 71.6 | 296.1 | 69.6 | 366.7 | 71.7 | 278.3 | 59.3 |
| ∑MUFA | 200.7 | 50.1 | 251.4 | 68.1 | 119.8 | 25.7 | 282.6 | 250.0 | 185.9 | 78.7 | 272.7 | 41.4 | 146.7 | 38.1 | 295.8 | 37.6 | 145.8 | 35.5 | 204.7 | 49.3 | 142.4 | 31.8 |
| ∑PUFA | 311.1 | 103.8 | 342.5 | 83.5 | 222.3 | 58.1 | 259.2 | 179.7 | 267.4 | 121.9 | 419.5 | 58.1 | 248.0 | 73.2 | 455.6 | 66.4 | 255.4 | 70.0 | 259.7 | 47.2 | 232.5 | 54.8 |
| ∑n-3 | 139.0 | 43.7 | 170.7 | 43.9 | 76.8 | 21.7 | 154.1 | 86.9 | 87.3 | 42.8 | 188.1 | 22.3 | 82.1 | 25.8 | 206.7 | 29.4 | 86.2 | 24.8 | 130.4 | 23.7 | 77.6 | 19.7 |
| ∑n-6 | 171.2 | 60.2 | 170.1 | 39.8 | 142.2 | 35.3 | 102.9 | 91.4 | 175.2 | 77.0 | 229.9 | 36.8 | 161.4 | 46.0 | 247.0 | 38.0 | 165.1 | 43.9 | 128.2 | 24.7 | 150.7 | 34.1 |
| ∑Other FA | 502 | 164 | 519 | 143 | 284 | 77 | 414 | 194 | 404 | 174 | 607 | 101 | 393 | 117 | 728 | 107 | 355 | 96 | 368 | 61 | 354 | 88 |

[1] % lipid is the percentage lipid in raw tissue; ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9,

22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.9** Sire breed and sex interactions with plane of nutrition on liver tissue fatty acid content [1]

| (mg/100g) | Black Suffolk | | Dorset | | | | Merino | | | | White Suffolk | | | | Ewe | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High (*n* 6) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 2) | | Low (*n* 4) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 9) | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | M |
| % lipid | 5.6 | 0.3 | 5.2 | 0.2 | 5.4 | 0.1 | 7.0 | 0.5 | 7.5 | 0.8 | 5.0 | 0.5 | 5.7 | 0.2 | 5.3 | 0.4 | |
| 14:0 | 14.8 | 1.7 | 7.9 | 2.7 | 6.9 | 2.4 | 18.2 | 18.2 | 14.5 | 9.4 | 9.1 | 4.1 | 6.9 | 2.9 | 7.9 | 2.7 | |
| 15:0 | 11.5 | 0.9 | 9.0 | 1.9 | 7.8 | 1.2 | 12.1 | 4.9 | 13.7 | 2.6 | 9.7 | 2.1 | 8.6 | 1.9 | 8.6 | 1.5 | |
| 16:1ω9c | 13.5 | 1.5 | 8.8 | 2.2 | 6.4 | 1.3 | 15.4 | 10.8 | 12.6 | 2.2 | 9.6 | 2.3 | 7.1 | 1.5 | 10.5 | 2.1 | |
| 16:1ω7c | 21.2 | 2.9 | 14.1 | 3.1 | 7.7 | 1.4 | 19.0 | 12.5 | 9.1 | 2.3 | 15.0 | 3.6 | 7.2 | 1.5 | 16.9 | 3.3 | |
| 16:0 | 530.6 | 43.8 | 464.6 | 82.0 | 298.0 | 49.6 | 595.6 | 219.9 | 477.8 | 75.5 | 430.8 | 72.7 | 330.6 | 76.9 | 442.5 | 65.4 | 3 |
| 17:0 | 39.0 | 2.4 | 30.6 | 5.0 | 25.6 | 4.5 | 36.3 | 11.9 | 32.9 | 6.8 | 27.6 | 4.4 | 27.1 | 6.2 | 28.8 | 4.4 | |
| 18:2ω6 | 173.5 | 5.4 | 180.3 | 56.0 | 85.3 | 9.2 | 232.2 | 56.0 | 142.4 | 24.1 | 140.3 | 17.5 | 88.6 | 15.8 | 144.5 | 18.6 | |
| 18:3ω3 | 101.3 | 11.5 | 97.2 | 19.2 | 30.5 | 2.7 | 107.1 | 51.8 | 68.0 | 19.5 | 98.5 | 22.1 | 31.3 | 4.8 | 90.4 | 15.9 | |
| 18:1ω9 | 702.8 | 70.2 | 551.8 | 101.8 | 336.0 | 61.2 | 735.5 | 386.0 | 448.9 | 64.5 | 503.6 | 100.3 | 344.9 | 80.0 | 573.7 | 100.2 | 3 |
| 18:1ω7c | 44.3 | 2.4 | 32.5 | 6.8 | 17.7 | 2.8 | 38.7 | 9.6 | 22.3 | 3.7 | 34.8 | 5.4 | 17.5 | 3.8 | 33.6 | 5.6 | |
| 18:1ω7t | 75.8 | 20.4 | 62.5 | 17.0 | 27.8 | 5.5 | 96.4 | 63.9 | 39.8 | 7.8 | 55.9 | 21.2 | 32.0 | 6.6 | 65.4 | 17.0 | |
| 18:0 | 758.4 | 24.9 | 663.5 | 97.9 | 311.9 | 66.8 | 680.4 | 219.5 | 335.7 | 88.8 | 633.5 | 97.5 | 314.8 | 76.8 | 629.7 | 84.3 | 2 |
| 20:4ω6 | 110.7 | 5.9 | 134.1 | 48.7 | 58.9 | 6.1 | 150.5 | 54.5 | 39.4 | 19.3 | 102.6 | 6.1 | 38.0 | 9.6 | 99.9 | 13.2 | |
| 20:5ω3 | 120.1 | 7.0 | 122.0 | 33.2 | 21.8 | 4.0 | 140.1 | 36.0 | 16.3 | 9.3 | 108.6 | 10.9 | 14.9 | 5.2 | 99.2 | 14.3 | |
| 20:3ω6 | 15.6 | 2.7 | 15.0 | 3.8 | 7.0 | 0.5 | 11.6 | 4.5 | 6.0 | 1.3 | 14.5 | 2.0 | 6.4 | 1.1 | 12.6 | 2.1 | |
| 20:4ω3 | 0.9 | 0.4 | 2.3 | 1.8 | 0.2 | 0.1 | 0.0 | 0.0 | 0.2 | 0.1 | 1.9 | 1.1 | 0.1 | 0.1 | 0.5 | 0.3 | |
| 20:2ω6 | 0.0 | 0.0 | 0.7 | 0.7 | 0.3 | 0.2 | 0.0 | 0.0 | 1.3 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| 20:0 | 3.1 | 0.4 | 3.6 | 1.6 | 1.6 | 0.9 | 4.1 | 2.3 | 1.0 | 0.8 | 2.9 | 0.3 | 0.9 | 0.4 | 2.5 | 0.4 | |
| 22:5ω6 | 0.0 | 0.0 | 0.3 | 0.3 | 2.9 | 1.1 | 0.0 | 0.0 | 3.5 | 2.0 | 0.2 | 0.2 | 12.6 | 9.1 | 0.0 | 0.0 | |
| 22:6ω3 | 98.8 | 7.3 | 102.4 | 26.2 | 49.0 | 6.4 | 54.4 | 1.3 | 27.2 | 11.1 | 106.2 | 18.5 | 39.9 | 6.9 | 94.9 | 17.7 | |
| 22:5ω3 | 130.0 | 15.5 | 116.8 | 25.2 | 32.9 | 3.6 | 106.5 | 14.1 | 26.3 | 12.3 | 120.5 | 18.0 | 30.1 | 4.7 | 108.3 | 17.3 | |
| 22:0 | 3.2 | 1.0 | 11.1 | 7.9 | 1.4 | 0.8 | 17.5 | 17.5 | 1.0 | 1.0 | 5.7 | 2.3 | 1.2 | 0.6 | 5.1 | 1.6 | |
| 23:0 | 5.3 | 2.4 | 7.6 | 2.5 | 3.9 | 2.4 | 4.1 | 3.6 | 1.5 | 1.5 | 7.7 | 2.2 | 2.7 | 1.4 | 5.0 | 1.7 | |
| 24:0 | 5.5 | 1.4 | 12.0 | 6.7 | 2.4 | 1.5 | 11.0 | 11.0 | 1.0 | 1.0 | 7.5 | 2.0 | 1.6 | 0.8 | 5.9 | 1.5 | |
| SUM | 2980.0 | 166.4 | 2650.6 | 477.6 | 1343.9 | 190.3 | 3086.7 | 951.0 | 1742.3 | 281.7 | 2446.8 | 381.3 | 1365.1 | 273.1 | 2486.4 | 363.1 | 13 |
| ∑SFA | 1392.3 | 72.1 | 1228.5 | 198.6 | 669.6 | 125.7 | 1404.3 | 447.4 | 892.6 | 172.1 | 1151.2 | 177.4 | 704.9 | 167.3 | 1152.9 | 159.1 | 6 |
| ∑MUFA | 957.1 | 80.7 | 752.6 | 138.5 | 435.5 | 78.0 | 1003.2 | 499.7 | 586.9 | 83.4 | 699.0 | 137.4 | 446.4 | 102.6 | 779.1 | 129.7 | 4 |
| ∑PUFA | 773.6 | 41.7 | 788.8 | 187.2 | 297.4 | 25.0 | 829.0 | 38.8 | 339.5 | 89.4 | 710.4 | 85.2 | 270.1 | 36.9 | 670.0 | 95.6 | 2 |
| ∑n-3 | 463.3 | 33.2 | 488.6 | 94.1 | 136.1 | 15.2 | 416.9 | 40.0 | 138.3 | 50.8 | 444.2 | 65.8 | 117.3 | 19.3 | 403.7 | 63.7 | 1 |
| ∑n-6 | 305.3 | 10.2 | 335.4 | 108.6 | 158.2 | 13.7 | 399.7 | 2.3 | 195.2 | 39.3 | 262.0 | 23.6 | 149.2 | 20.6 | 261.9 | 33.0 | 1 |
| ∑Other FA | 143.0 | 10.9 | 119.3 | 23.1 | 58.5 | 10.2 | 149.7 | 34.9 | 76.7 | 14.3 | 113.9 | 17.8 | 56.4 | 14.1 | 115.5 | 17.1 | |

[1] % lipid is the percentage lipid in raw tissue; ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S9.10** Sire breed and sex interactions with plane of nutrition on *Longissimus dorsi* muscle tissue fatty acid content [1]

| (mg/100g) | Black Suffolk | | Dorset | | | | Merino | | | | White Suffolk | | | | Ewe | | | | Wether | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High (*n* 6) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 2) | | Low (*n* 4) | | High (*n* 6) | | Low (*n* 8) | | High (*n* 9) | | Low (*n* 8) | | High (*n* 11) | | Low (*n* 12) | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| % lipid | 4.3 | 0.4 | 4.6 | 0.4 | 3.9 | 0.5 | 4.5 | 0.5 | 4.7 | 0.6 | 5.4 | 0.5 | 3.6 | 0.8 | 4.9 | 0.4 | 4.0 | 0.9 | 6.3 | 0.3 | 3.9 | 0.3 |
| 14:0 | 62.7 | 14.2 | 79.7 | 13.0 | 36.6 | 16.5 | 66.2 | 21.8 | 24.5 | 16.4 | 74.9 | 2.0 | 20.6 | 7.9 | 77.3 | 4.7 | 34.9 | 4.5 | 67.4 | 0.2 | 23.0 | 9.0 |
| 15:0 | 14 | 2. | 17 | 3. | 8. | 3. | 16 | 2. | 4. | 0. | 17 | 3. | 3. | 0. | 16 | 2. | 6. | 3. | 15 | 2. | 5. | 1. |

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | .0 | 5 | .0 | 2 | 3 | 6 | .7 | 5 | 6 | 8 | .2 | 8 | 7 | 8 | .8 | 7 | 8 | 0 | .6 | 1 | 0 | 6 |
| 16:1 | 8. | 1. | 10 | 2. | 5. | 1. | 9. | 1. | 3. | 1. | 10 | 2. | 3. | 0. | 10 | 1. | 4. | 1. | 9. | 1. | 3. | 0. |
| ω9c | 8 | 6 | .0 | 0 | 1 | 8 | 6 | 4 | 9 | 5 | .7 | 4 | 1 | 7 | .5 | 7 | 9 | 7 | 3 | 2 | 5 | 8 |
| 16:1 | 35 | 7. | 45 | 5. | 24 | 7. | 31 | 8. | 17 | 8. | 38 | 7. | 11 | 5. | 42 | 6. | 22 | 6. | 36 | 3. | 14 | 4. |
| ω7c | .6 | 4 | .6 | 8 | .1 | 0 | .3 | 7 | .8 | 2 | .2 | 4 | .8 | 1 | .2 | 7 | .9 | 5 | .3 | 7 | .6 | 7 |
| 16:0 | 63 | 11 | 86 | 10 | 45 | 15 | 63 | 13 | 31 | 13 | 76 | 17 | 26 | 66 | 78 | 11 | 43 | 14 | 71 | 85 | 29 | 76 |
| | 8. | 18. | 7. | 03. | 0. | 57. | 9. | 37. | 3. | 35. | 8. | 2. | 5. | 2. | 6. | 18. | 0. | 4 | 3. | 5. | 4. | 0. |
| | 8 | 1 | 7 | 4 | 8 | 9 | 7 | 6 | 3 | 6 | 2 | 0 | 4 | 2 | 7 | 6 | 8 | 19 | 4 | 7 | 7 | 0 |
| 17:0 | 43 | 7. | 46 | 6. | 26 | 9. | 44 | 6. | 19 | 7. | 41 | 9. | 16 | 3. | 44 | 7. | 25 | 8. | 43 | 4. | 18 | 4. |
| | .9 | 9 | .8 | 8 | .9 | 0 | .5 | 6 | .2 | 7 | .1 | 4 | .1 | 6 | .3 | 5 | .5 | 1 | .7 | 3 | .0 | 4 |
| 18:2 | 98 | 15. | 13 | 23. | 68 | 16. | 16 | 33. | 52 | 14. | 93 | 15. | 57 | 14. | 10 | 13. | 73 | 11 | 12 | 16. | 52 | 10. |
| ω6 | .5 | 4 | 0. | 6 | .9 | 4 | 7. | 4 | .0 | 3 | .5 | 5 | .1 | 4 | 2. | 23 | .0 | 5. | 2. | 4 | .7 | 8 |
| | | | 1 | | | | 7 | | | | | 5 | | 4 | 2 | | | 1 | 6 | | | |
| 18:3 | 59 | 10. | 73 | 7. | 29 | 8. | 83 | 9. | 23 | 8. | 70 | 12. | 20 | 3. | 67 | 9. | 28 | 7. | 71 | 6. | 21 | 4. |
| ω3 | .8 | 1 | .6 | 3 | .2 | 0 | .4 | 8 | .3 | 3 | .5 | 9 | .0 | 8 | .3 | 4 | .4 | 2 | .3 | 3 | .7 | 3 |
| 18:1 | 95 | 15 | 13 | 14 | 72 | 23 | 10 | 17 | 44 | 18 | 11 | 22 | 42 | 91 | 11 | 18 | 67 | 20 | 11 | 10 | 46 | 11 |
| ω9 | 7. | 7. | 90 | 44. | 9. | 2. | 11 | 3. | 5. | 5. | 36 | 4. | 8. | .1 | 50 | 0. | 8. | 7. | 42 | 4. | 7. | 13. |
| | 0 | 2 | .0 | 7 | 1 | 7 | .6 | 3 | 0 | 8 | .9 | 8 | 5 | 4 | .9 | 4 | 3 | 9 | .6 | 8 | 9 | 5 |
| 18:1 | 46 | 7. | 49 | 6. | 25 | 7. | 37 | 5. | 17 | 6. | 49 | 10. | 16 | 3. | 48 | 6. | 24 | 6. | 46 | 5. | 18 | 3. |
| ω7c | .4 | 6 | .5 | 2 | .5 | 3 | .0 | 2 | .9 | 7 | .6 | 7 | .8 | 3 | .5 | 9 | .3 | 5 | .4 | 4 | .0 | 8 |
| 18:1 | 89 | 13. | 10 | 14. | 51 | 19. | 11 | 28. | 33 | 13. | 10 | 23. | 30 | 6. | 10 | 14. | 48 | 18. | 99 | 12. | 33 | 7. |
| ω7t | .4 | 6 | 3. | 9 | .7 | 2 | 6. | 7 | .3 | 5 | 5. | 5 | .4 | 2 | 3. | 3 | .3 | 4 | .2 | 4 | .6 | 3 |
| | | | 8 | | | | 0 | | | | 6 | | | | 8 | | | | | | | |
| 18:0 | 53 | 9 | 80 | 12 | 44 | 17 | 70 | 10 | 25 | 11 | 71 | 17 | 21 | 42. | 69 | 12. | 39 | 15. | 67 | 8 | 26 | 7 |
| | 1. | 0. | 4. | 23. | 8. | 07. | 2. | 07. | 7. | 10. | 0. | 7 | 1. | 2. | 9. | 2. | 2. | 4. | 2. | 7. | 3. | 33. |
| | 2 | 6 | 3 | 7 | 3 | 8 | 6 | 6 | 3 | 4 | 9 | 7. | 1 | 7 | 0 | 1. | 4 | 4. | 1 | 3 | 7 | 3 |
| | | | | | | | | | | | | 4 | | | | 5 | | 4 | | | | |
| 20:4 | 12 | 4. | 17 | 3. | 12 | 4. | 27 | 1. | 7. | 1. | 9. | 2. | 11 | 3. | 13 | 2. | 14 | 3. | 15 | 3. | 9. | 2. |
| ω6 | .7 | 2 | .1 | 5 | .6 | 0 | .6 | 6 | 6 | 9 | 7 | 2 | .9 | 6 | .0 | 7 | .1 | 9 | .9 | 0 | 4 | 4 |
| 20:5 | 13 | 5. | 15 | 3. | 6. | 2. | 19 | 8. | 3. | 1. | 8. | 3. | 6. | 1. | 14 | 3. | 7. | 2. | 12 | 3. | 4. | 1. |
| ω3 | .5 | 5 | .7 | 9 | 9 | 3 | .7 | 4 | 5 | 2 | 8 | 7 | 4 | 9 | .8 | 5 | 8 | 2 | .2 | 4 | 8 | 3 |
| 20:3 | 0. | 0. | 3. | 1. | 3. | 0. | 6. | 0. | 1. | 0. | 2. | 0. | 2. | 0. | 1. | 0. | 3. | 0. | 3. | 1. | 1. | 0. |
| ω6 | 7 | 7 | 5 | 8 | 4 | 9 | 9 | 3 | 2 | 5 | 2 | 8 | 5 | 7 | 2 | 7 | 5 | 8 | 8 | 1 | 9 | 5 |
| 20:4 | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 1. | 1. | 0. | 0. | 0. | 0. | 1. | 0. | 1. | 0. | 0. | 0. |
| ω3 | 1 | 1 | 0 | 0 | 8 | 4 | 5 | 5 | 1 | 1 | 6 | 1 | 8 | 4 | 1 | 1 | 0 | 5 | 0 | 6 | 4 | 2 |
| 20:2 | 0. | 0. | 0. | 0. | 1. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 1. | 0. | 0. | 0. | 0. | 0. |
| ω6 | 1 | 1 | 0 | 0 | 3 | 5 | 2 | 2 | 1 | 1 | 1 | 1 | 3 | 2 | 2 | 1 | 0 | 5 | 0 | 0 | 4 | 2 |
| 20:0 | 1. | 1. | 1. | 0. | 3. | 1. | 3. | 0. | 1. | 0. | 0. | 0. | 1. | 0. | 0. | 0. | 2. | 1. | 1. | 0. | 1. | 0. |
| | 2 | 2 | 4 | 9 | 2 | 2 | 6 | 6 | 4 | 9 | 4 | 4 | 4 | 4 | 8 | 8 | 8 | 0 | 6 | 6 | 6 | 6 |
| 22:5 | 0. | 0. | 0. | 0. | 1. | 0. | 0. | 0. | 9. | 8. | 0. | 0. | 0. | 0. | 0. | 0. | 1. | 0. | 0. | 0. | 3. | 2. |
| ω6 | 0 | 0 | 0 | 0 | 5 | 7 | 0 | 0 | 3 | 7 | 0 | 0 | 8 | 6 | 0 | 0 | 7 | 8 | 0 | 0 | 5 | 9 |
| 22:6 | 0. | 0. | 1. | 1. | 3. | 1. | 6. | 1. | 0. | 0. | 0. | 0. | 2. | 1. | 0. | 0. | 3. | 1. | 2. | 0. | 1. | 0. |
| ω3 | 8 | 8 | 7 | 1 | 1 | 1 | 6 | 2 | 5 | 4 | 7 | 7 | 5 | 0 | 6 | 6 | 4 | 1 | 5 | 9 | 6 | 7 |
| 22:5 | 3. | 3. | 5. | 3. | 8. | 2. | 21 | 2. | 3. | 1. | 3. | 3. | 6. | 2. | 2. | 2. | 8. | 2. | 8. | 3. | 5. | 1. |
| ω3 | 7 | 7 | 4 | 5 | 4 | 9 | .9 | 1 | 1 | 3 | 0 | 0 | 2 | 0 | 5 | 4 | 4 | 4 | 5 | 1 | 2 | 8 |
| 22:0 | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. |
| | 1 | 1 | 1 | 1 | 5 | 3 | 5 | 5 | 0 | 0 | 8 | 7 | 4 | 2 | 6 | 5 | 5 | 3 | 2 | 1 | 3 | 1 |
| 23:0 | 0. | 0. | 0. | 0. | 0. | 0. | 1. | 1. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. |
| | 1 | 1 | 0 | 0 | 7 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 5 | 3 | 1 | 1 | 6 | 3 | 2 | 2 | 3 | 2 |
| 24:0 | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. | 0. |
| | 2 | 2 | 0 | 0 | 6 | 3 | 5 | 5 | 0 | 0 | 2 | 2 | 4 | 3 | 1 | 1 | 6 | 3 | 2 | 1 | 2 | 2 |
| SU | 26 | 41 | 36 | 41 | 19 | 65 | 30 | 53 | 12 | 52 | 31 | 67 | 11 | 24 | 31 | 47 | 18 | 57 | 30 | 32 | 12 | 31 |
| M | 19 | 9. | 62 | 2. | 47 | 0. | 15 | 6. | 39 | 2. | 44 | 4. | 18 | 0. | 83 | 8. | 16 | 8. | 85 | 1. | 46 | 2. |
| | .4 | 6 | .8 | 1 | .4 | 1 | .7 | 6 | .1 | 0 | .9 | 1 | .7 | 3 | .2 | 2 | .3 | 8 | .9 | 4 | .3 | 2 |
| ∑SF | 13 | 23 | 18 | 25 | 98 | 36 | 14 | 26 | 62 | 27 | 16 | 35 | 52 | 11 | 16 | 29 | 90 | 37 | 15 | 11 | 61 | 11 |
| A | 14 | 5. | 44 | 1. | 8. | 2. | 85 | 8. | 8. | 3. | 41 | 8. | 8. | 84 | 52 | 6. | 7. | 4. | 36 | 8 | 5. | 16 |
| | .7 | 8 | .8 | 9 | 3 | 3 | .8 | 9 | 0 | 8 | .3 | 8. | 4 | 8. | .9 | 0 | 8 | 1. | .4 | 9. | 2 | 6. |
| | | | | | | | | | | | | 2 | | 1 | | | | 4 | | 7 | | 0 |
| ∑M | 12 | 19 | 17 | 18 | 89 | 28 | 12 | 21 | 55 | 23 | 14 | 22 | 52 | 11 | 14 | 22 | 83 | 25 | 14 | 13 | 57 | 13 |
| UFA | 39 | 2. | 20 | 2. | 5. | 3. | 99 | 6. | 6. | 1. | 71 | 7 | 8. | 22 | 79 | 1 | 6. | 3. | 42 | 4. | 8. | 8. |
| | .6 | 2 | .0 | 8 | 9 | 7 | .2 | 4 | 9 | 4 | .2 | 8. | 7 | 5 | .5 | 3. | 0 | 3. | .5 | 3 | 0 | 9 |
| | | | | | | | | | | | | 3 | | | | 7 | | 9 | | | | |
| ∑PU | 19 | 36 | 25 | 38 | 14 | 34 | 34 | 41 | 10 | 37 | 19 | 30 | 11 | 28 | 21 | 29 | 14 | 31 | 24 | 28 | 10 | 23 |
| FA | 8. | 6. | 8. | 3. | 1. | 4. | 3. | 2. | 4. | 4. | 9. | 2. | 3. | 5. | 2. | 7. | 8. | 2. | 6. | 2 | 5. | 3. |
| | 5 | 6 | 3 | 3 | 9 | 4 | 6 | 2 | 3 | 4 | 4 | 2 | 0 | 3 | 0 | 7 | 4 | 2 | 9 | 2 | 8 | 0 |
| ∑n- | 77 | 16. | 97 | 11 | 48 | 12. | 13 | 4. | 31 | 11 | 84 | 12. | 36 | 8. | 85 | 12. | 49 | 10. | 96 | 9. | 33 | 7. |
| 3 | .9 | 3 | .1 | 12 | .6 | 4 | 2. | 7 | .0 | 1. | .7 | 4 | .1 | 7 | .3 | 5. | .4 | 9 | .0 | 3 | .9 | 5 |
| | | | | | | | 8 | | | 1 | | | | | | | | | | | | |
| ∑n- | 11 | 19. | 15 | 28. | 88 | 21. | 20 | 35. | 70 | 25. | 10 | 16. | 73 | 19. | 11 | 16. | 94 | 19. | 14 | 19. | 68 | 14. |
| 6 | 3. | 6 | 3. | 0 | .6 | 0 | 4. | 2 | .3 | 2 | 6. | 4 | .1 | 0 | 8. | 2 | .2 | 7 | 3. | 7 | .4 | 8 |
| | 4 | | 1 | | | | 2 | | | | 6 | | | | 9 | | | | 4 | | | |
| ∑Ot | 13 | 26. | 16 | 17. | 78 | 21. | 11 | 10. | 50 | 20. | 16 | 20. | 51 | 10. | 16 | 18. | 75 | 18. | 13 | 14. | 52 | 12. |
| her | 3. | 1 | 0. | 0 | .7 | 7 | 2. | 1 | .1 | 0 | 7. | 6 | .4 | 7 | 1. | 6 | .9 | 5 | 9. | 0 | .8 | 1 |
| FA | 3 | | 3 | | | | 9 | | | | 0 | | | | 3 | | | | 9 | | | |

[1] % lipid is the percentage lipid in raw tissue; ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9,

22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## Chapter 10: Fatty acid profile: sire breed and sex

**S10.1** Sire breed and sex interactions on subcutaneous adipose tissue fatty acid composition [1]

| *(% total FA)* | Black Suffolk | | | | Dorset | | | | Merino | | White Suffolk (n35) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe | | Wether | | Ewe | | Wether | | Wether | | Ewe | | Wether | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 3.0 | 0.7 | 2.0 | 1.0 | 2.1 | 0.5 | 2.7 | 0.3 | 3.3 | 0.4 | 2.9 | 0.3 | 2.6 | 0.5 |
| 15:0 | 0.7 | 0.1 | 0.6 | 0.3 | 0.6 | 0.1 | 0.7 | 0.1 | 0.7 | 0.1 | 0.7 | 0.1 | 0.6 | 0.1 |
| 16:1ω9c | 0.4 | 0.0 | 0.4 | 0.1 | 0.3 | 0.1 | 0.3 | 0.0 | 0.4 | 0.0 | 0.3 | 0.0 | 0.4 | 0.0 |
| 16:1ω7c | 1.5 | 0.1 | 1.1 | 0.2 | 1.0 | 0.2 | 1.1 | 0.3 | 0.7 | 0.2 | 1.2 | 0.2 | 1.4 | 0.2 |
| 16:0 | 23.7 | 1.0 | 24.5 | 0.4 | 23.5 | 1.5 | 23.7 | 0.5 | 24.4 | 1.0 | 24.2 | 0.9 | 23.6 | 0.5 |
| 17:0 | 2.2 | 0.3 | 2.7 | 0.1 | 1.9 | 0.1 | 1.8 | 0.1 | 2.0 | 0.1 | 1.8 | 0.1 | 1.7 | 0.1 |
| 18:2ω6 | 1.6 | 0.0 | 1.7 | 0.2 | 1.5 | 0.2 | 1.4 | 0.1 | 2.1 | 0.2 | 1.5 | 0.1 | 1.5 | 0.0 |
| 18:3ω3 | 1.3 | 0.1 | 1.6 | 0.2 | 1.2 | 0.2 | 1.3 | 0.1 | 1.6 | 0.1 | 1.4 | 0.2 | 1.4 | 0.1 |
| 18:1ω9 | 35.2 | 1.4 | 30.5 | 1.1 | 29.8 | 3.2 | 35.7 | 2.1 | 29.0 | 1.8 | 34.5 | 1.6 | 34.6 | 1.6 |
| 18:1ω7c | 1.7 | 0.1 | 1.5 | 0.2 | 1.0 | 0.2 | 1.3 | 0.1 | 1.2 | 0.0 | 1.3 | 0.1 | 1.3 | 0.0 |
| 18:1ω7t | 4.5 | 0.7 | 4.2 | 0.1 | 3.0 | 0.2 | 3.3 | 0.4 | 3.6 | 0.5 | 3.5 | 0.4 | 3.9 | 0.3 |
| 18:0 | 18.9 | 2.1 | 23.1 | 3.5 | 29.8 | 5.2 | 22.3 | 1.8 | 26.8 | 1.6 | 21.9 | 1.0 | 22.2 | 1.5 |
| 20:4ω6 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:5ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 |
| 20:3ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| 20:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.3 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:6ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.2 |
| 22:5ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 23:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ∑SFA | 49.2 | 0.7 | 53.6 | 2.4 | 59.0 | 3.9 | 52.0 | 2.1 | 58.0 | 1.8 | 52.3 | 1.4 | 51.6 | 1.8 |
| ∑MUFA | 47.5 | 0.6 | 42.9 | 2.0 | 37.8 | 3.8 | 44.7 | 2.2 | 37.7 | 1.9 | 44.1 | 1.5 | 44.7 | 1.7 |
| ∑PUFA | 3.2 | 0.2 | 3.5 | 0.5 | 3.1 | 0.5 | 3.3 | 0.2 | 4.3 | 0.3 | 3.6 | 0.4 | 3.7 | 0.3 |
| ∑n-3 | 1.3 | 0.1 | 1.6 | 0.2 | 1.3 | 0.2 | 1.5 | 0.1 | 1.7 | 0.1 | 1.6 | 0.2 | 1.8 | 0.3 |
| ∑n-6 | 1.6 | 0.0 | 1.8 | 0.3 | 1.6 | 0.3 | 1.6 | 0.1 | 2.4 | 0.2 | 1.7 | 0.1 | 1.7 | 0.1 |
| ∑Other FA | 5.2 | 0.4 | 6.0 | 0.6 | 4.0 | 0.5 | 4.1 | 0.3 | 3.8 | 0.2 | 4.2 | 0.3 | 4.1 | 0.3 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S10.2** Sire breed and sex interactions on heart tissue fatty acid composition [1]

| *(% total FA)* | Black Suffolk | | | | Dorset | | | | Merino | | White Suffolk (n35) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe | | Wether | | Ewe | | Wether | | Wether | | Ewe | | Wether | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 0.9 | 0.1 | 0.7 | 0.3 | 1.0 | 0.2 | 0.7 | 0.2 | 1.1 | 0.3 | 0.6 | 0.2 | 0.4 | 0.1 |
| 15:0 | 0.4 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 | 0.4 | 0.0 | 0.3 | 0.1 | 0.3 | 0.1 | 0.4 | 0.1 |
| 16:1ω9c | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 |
| 16:1ω7c | 0.5 | 0.0 | 0.4 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.4 | 0.1 | 0.3 | 0.1 | 0.4 | 0.1 |
| 16:0 | 14.0 | 0.5 | 12.7 | 0.6 | 14.6 | 0.6 | 15.6 | 0.6 | 16.5 | 0.8 | 14.9 | 0.4 | 13.6 | 0.3 |
| 17:0 | 1.4 | 0.1 | 1.3 | 0.1 | 1.4 | 0.1 | 1.4 | 0.0 | 1.4 | 0.1 | 1.3 | 0.1 | 1.3 | 0.0 |
| 18:2ω6 | 14.8 | 0.9 | 17.5 | 3.2 | 14.5 | 1.6 | 13.9 | 1.7 | 17.5 | 2.1 | 16.6 | 1.9 | 19.3 | 1.2 |
| 18:3ω3 | 3.2 | 0.2 | 4.1 | 0.5 | 2.7 | 0.3 | 2.8 | 0.4 | 3.3 | 0.4 | 2.8 | 0.4 | 3.9 | 0.5 |
| 18:1ω9 | 20.8 | 2.0 | 17.1 | 2.6 | 20.2 | 1.3 | 19.1 | 1.1 | 20.2 | 0.9 | 18.7 | 1.1 | 17.6 | 0.4 |
| 18:1ω7c | 2.0 | 0.1 | 1.9 | 0.1 | 2.0 | 0.1 | 1.8 | 0.1 | 2.0 | 0.1 | 2.0 | 0.1 | 2.0 | 0.1 |
| 18:1ω7t | 2.8 | 0.3 | 2.4 | 0.2 | 2.0 | 0.4 | 2.1 | 0.3 | 2.2 | 0.2 | 2.0 | 0.3 | 1.8 | 0.2 |
| 18:0 | 21.2 | 0.5 | 21.0 | 1.6 | 22.1 | 1.9 | 22.3 | 1.9 | 20.1 | 1.7 | 20.7 | 1.7 | 18.8 | 1.3 |
| 20:4ω6 | 4.5 | 0.5 | 4.8 | 0.4 | 4.4 | 0.9 | 5.0 | 1.0 | 4.1 | 0.6 | 5.2 | 0.9 | 5.4 | 0.5 |
| 20:5ω3 | 3.1 | 0.5 | 3.3 | 0.5 | 1.8 | 0.2 | 2.9 | 1.0 | 1.9 | 0.3 | 2.8 | 1.0 | 2.5 | 0.3 |
| 20:3ω6 | 0.5 | 0.0 | 0.6 | 0.0 | 0.5 | 0.1 | 0.5 | 0.1 | 0.5 | 0.1 | 0.6 | 0.1 | 0.7 | 0.0 |
| 20:4ω3 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 00 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:0 | 0.1 | 0.0 | 0.2 | 0.0 | 0.2 | 0.1 | 0.2 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.2 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:6ω3 | 0.7 | 0.1 | 0.9 | 0.2 | 1.0 | 0.2 | 1.1 | 0.3 | 0.7 | 0.1 | 1.1 | 0.3 | 1.1 | 0.1 |
| 22:5ω3 | 1.1 | 0.6 | 1.9 | 0.3 | 1.4 | 0.3 | 1.6 | 0.3 | 1.0 | 0.2 | 1.7 | 0.5 | 1.8 | 0.2 |
| 22:0 | 0.1 | 0.1 | 0.2 | 0.0 | 0.2 | 0.0 | 0.4 | 0.2 | 0.1 | 0.0 | 0.3 | 0.2 | 0.2 | 0.0 |
| 23:0 | 0.1 | 0.1 | 0.3 | 0.0 | 0.3 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 |
| 24:0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.2 | 0.0 | 0.2 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 | 0.2 | 0.0 |
| ∑SFA | 39.2 | 0.4 | 37.8 | 2.4 | 41.2 | 2.5 | 42.3 | 2.4 | 40.9 | 2.6 | 39.6 | 2.0 | 36.1 | 1.4 |
| ∑MUFA | 32.5 | 1.9 | 28.5 | 2.0 | 31.1 | 1.3 | 29.2 | 1.5 | 29.5 | 1.1 | 28.5 | 1.7 | 28.3 | 0.4 |
| ∑PUFA | 28.3 | 1.8 | 33.7 | 3.9 | 27.7 | 3.2 | 28.6 | 2.9 | 29.6 | 3.3 | 31.8 | 2.8 | 35.6 | 1.7 |
| ∑n-3 | 8.1 | 1.0 | 10.3 | 1.5 | 7.1 | 0.7 | 8.5 | 1.5 | 7.1 | 0.7 | 8.7 | 1.6 | 9.5 | 0.8 |
| ∑n-6 | 19.9 | 1.2 | 23.1 | 3.2 | 19.9 | 2.6 | 19.6 | 2.0 | 22.2 | 2.7 | 22.5 | 2.2 | 25.6 | 1.7 |
| ∑Other FA | 7.6 | 0.9 | 7.8 | 0.7 | 8.4 | 1.6 | 7.2 | 0.6 | 6.0 | 0.5 | 7.2 | 0.7 | 8.0 | 0.3 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S10.3** Sire breed and sex interactions on kidney tissue fatty acid composition [1]

| *(% total FA)* | Black Suffolk | | | | Dorset | | | | Merino | | White Suffolk (n35) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe | | Wether | | Ewe | | Wether | | Wether | | Ewe | | Wether | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.2 | 0.3 | 0.1 | 0.1 | 0.1 |
| 15:0 | 0.3 | 0.0 | 0.5 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 | 0.3 | 0.0 |
| 16:1ω9c | 0.2 | 0.0 | 0.1 | 0.1 | 0.2 | 0.0 | 0.2 | 0.1 | 0.3 | 0.1 | 0.2 | 0.0 | 0.1 | 0.0 |
| 16:1ω7c | 0.3 | 0.0 | 0.2 | 0.1 | 0.3 | 0.0 | 0.4 | 0.1 | 0.5 | 0.2 | 0.2 | 0.1 | 0.3 | 0.0 |
| 16:0 | 17.2 | 1.3 | 17.9 | 0.5 | 18.6 | 0.5 | 16.6 | 0.6 | 23.1 | 3.0 | 18.5 | 0.7 | 19.4 | 0.8 |
| 17:0 | 1.3 | 0.0 | 1.4 | 0.2 | 1.3 | 0.1 | 1.3 | 0.1 | 1.3 | 0.2 | 1.4 | 0.1 | 1.4 | 0.1 |
| 18:2ω6 | 7.8 | 0.4 | 10.1 | 1.7 | 9.4 | 0.6 | 9.3 | 0.7 | 9.6 | 1.1 | 9.5 | 0.4 | 9.6 | 0.3 |
| 18:3ω3 | 1.8 | 0.1 | 2.9 | 0.4 | 1.9 | 0.2 | 1.8 | 0.4 | 7.0 | 4.6 | 1.7 | 0.3 | 1.9 | 0.4 |
| 18:1ω9 | 14.7 | 1.2 | 20.2 | 5.1 | 14.7 | 0.5 | 16.1 | 1.5 | 15.8 | 2.7 | 15.6 | 0.5 | 14.3 | 0.7 |
| 18:1ω7c | 1.3 | 0.1 | 0.9 | 0.4 | 1.4 | 0.1 | 1.6 | 0.2 | 1.7 | 0.3 | 1.5 | 0.1 | 1.6 | 0.2 |
| 18:1ω7t | 1.4 | 0.6 | 2.6 | 0.9 | 1.2 | 0.1 | 1.2 | 0.3 | 1.1 | 0.4 | 1.2 | 0.3 | 1.2 | 0.2 |
| 18:0 | 20.4 | 0.8 | 22.9 | 0.6 | 20.9 | 0.8 | 20.4 | 1.7 | 18.3 | 3.5 | 20.8 | 1.4 | 20.6 | 1.3 |
| 20:4ω6 | 10.5 | 1.5 | 4.6 | 2.3 | 9.4 | 1.3 | 10.9 | 1.7 | 5.6 | 1.7 | 10.2 | 1.1 | 9.3 | 1.4 |
| 20:5ω3 | 7.1 | 0.2 | 5.6 | 2.8 | 5.2 | 1.2 | 5.4 | 0.9 | 2.2 | 0.7 | 5.0 | 0.8 | 5.1 | 1.1 |
| 20:3ω6 | 0.6 | 0.0 | 0.5 | 0.3 | 0.7 | 0.1 | 0.7 | 0.1 | 0.5 | 0.1 | 0.7 | 0.1 | 0.6 | 0.1 |
| 20:4ω3 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 |
| 20:0 | 0.2 | 0.1 | 0.0 | 0.0 | 0.2 | 0.0 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 | 0.0 | 0.2 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.2 | 0.2 | 0.2 | 0.5 | 0.3 | 0.4 | 0.2 | 0.4 | 0.3 |
| 22:6ω3 | 2.5 | 0.3 | 1.4 | 0.7 | 3.5 | 0.3 | 3.3 | 0.4 | 1.9 | 0.4 | 2.5 | 0.3 | 2.9 | 0.3 |
| 22:5ω3 | 3.7 | 0.5 | 2.2 | 1.1 | 3.1 | 0.2 | 3.0 | 0.3 | 2.0 | 0.5 | 2.9 | 0.4 | 2.9 | 0.3 |
| 22:0 | 1.6 | 0.0 | 1.0 | 0.5 | 1.1 | 0.1 | 1.3 | 0.5 | 1.0 | 0.4 | 0.9 | 0.2 | 1.1 | 0.2 |
| 23:0 | 0.3 | 0.1 | 0.0 | 0.0 | 0.2 | 0.1 | 0.3 | 0.2 | 0.3 | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 |
| 24:0 | 1.1 | 0.1 | 0.5 | 0.3 | 0.8 | 0.2 | 1.2 | 0.6 | 0.9 | 0.4 | 0.7 | 0.2 | 0.9 | 0.2 |
| ∑SFA | 43.4 | 0.7 | 45.1 | 0.2 | 44.3 | 1.1 | 42.4 | 2.1 | 47.1 | 2.1 | 44.3 | 2.1 | 45.2 | 2.2 |
| ∑MUFA | 22.2 | 1.6 | 27.1 | 5.2 | 21.5 | 0.7 | 22.6 | 1.7 | 22.8 | 2.2 | 22.5 | 0.7 | 21.4 | 0.8 |
| ∑PUFA | 34.4 | 2.1 | 27.8 | 5.4 | 34.2 | 1.6 | 35.0 | 2.9 | 30.2 | 2.2 | 33.2 | 2.4 | 33.5 | 2.4 |
| ∑n-3 | 15.4 | 0.7 | 12.4 | 4.4 | 13.8 | 1.2 | 13.6 | 1.3 | 13.3 | 3.5 | 12.1 | 1.5 | 12.9 | 1.8 |
| ∑n-6 | 19.0 | 1.9 | 15.2 | 0.9 | 20.0 | 1.5 | 21.1 | 2.5 | 16.3 | 2.9 | 20.9 | 1.5 | 20.1 | 1.4 |
| ∑Other FA | 5.7 | 0.2 | 4.5 | 0.6 | 5.1 | 0.2 | 4.4 | 0.5 | 5.4 | 0.9 | 4.9 | 0.3 | 5.4 | 0.6 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

## **S10.4** Sire breed and sex interactions on liver tissue fatty acid composition [1]

| | Black Suffolk | | | | Dorset | | | | Merino | | White Suffolk (n35) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe | | Wether | | Ewe | | Wether | | Wether | | Ewe | | Wether | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 0.5 | 0.0 | 0.5 | 0.1 | 0.3 | 0.1 | 0.4 | 0.1 | 0.7 | 0.4 | 0.2 | 0.1 | 0.4 | 0.1 |
| 15:0 | 0.4 | 0.0 | 0.4 | 0.0 | 0.4 | 0.1 | 0.5 | 0.1 | 0.6 | 0.1 | 0.4 | 0.1 | 0.5 | 0.0 |
| 16:1ω9c | 0.5 | 0.0 | 0.4 | 0.0 | 0.4 | 0.1 | 0.4 | 0.1 | 0.6 | 0.1 | 0.4 | 0.1 | 0.4 | 0.0 |
| 16:1ω7c | 0.8 | 0.1 | 0.6 | 0.1 | 0.6 | 0.1 | 0.6 | 0.2 | 0.6 | 0.2 | 0.6 | 0.1 | 0.6 | 0.1 |
| 16:0 | 16.7 | 1.0 | 17.1 | 0.9 | 20.1 | 1.1 | 18.4 | 0.8 | 23.6 | 2.3 | 20.2 | 1.3 | 19.7 | 1.3 |
| 17:0 | 1.2 | 0.1 | 1.3 | 0.1 | 1.5 | 0.1 | 1.5 | 0.1 | 1.5 | 0.2 | 1.5 | 0.2 | 1.6 | 0.2 |
| 18:2ω6 | 5.5 | 0.4 | 5.7 | 0.3 | 5.8 | 0.6 | 6.6 | 0.9 | 7.7 | 0.4 | 6.9 | 0.5 | 5.6 | 0.5 |
| 18:3ω3 | 3.1 | 0.4 | 3.3 | 0.3 | 2.8 | 0.4 | 2.9 | 0.4 | 3.6 | 0.6 | 2.9 | 0.4 | 2.9 | 0.5 |
| 18:1ω9 | 23.3 | 1.7 | 21.2 | 1.2 | 22.9 | 1.0 | 21.1 | 1.3 | 23.7 | 1.8 | 22.7 | 2.0 | 20.9 | 0.9 |
| 18:1ω7c | 1.4 | 0.1 | 1.4 | 0.1 | 1.0 | 0.2 | 1.2 | 0.1 | 1.2 | 0.1 | 1.3 | 0.1 | 1.3 | 0.1 |
| 18:1ω7t | 2.7 | 1.2 | 2.2 | 0.9 | 2.4 | 0.3 | 1.8 | 0.3 | 2.3 | 0.3 | 1.9 | 0.4 | 2.3 | 0.4 |
| 18:0 | 23.0 | 0.7 | 26.0 | 1.0 | 25.0 | 3.7 | 22.2 | 2.2 | 18.7 | 1.8 | 21.8 | 1.6 | 24.0 | 1.8 |
| 20:4ω6 | 3.8 | 0.2 | 3.4 | 0.3 | 3.7 | 0.9 | 5.2 | 0.9 | 3.4 | 1.3 | 4.7 | 1.2 | 3.4 | 0.8 |
| 20:5ω3 | 3.7 | 0.0 | 4.0 | 0.2 | 2.1 | 0.6 | 3.3 | 0.8 | 2.4 | 1.2 | 3.2 | 0.8 | 2.5 | 0.7 |
| 20:3ω6 | 0.4 | 0.1 | 0.6 | 0.2 | 0.5 | 0.1 | 0.5 | 0.0 | 0.4 | 0.1 | 0.5 | 0.1 | 0.5 | 0.1 |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.8 | 0.7 |
| 22:6ω3 | 3.3 | 0.4 | 3.2 | 0.5 | 3.6 | 0.9 | 3.9 | 0.8 | 1.6 | 0.4 | 3.1 | 0.5 | 4.1 | 0.7 |
| 22:5ω3 | 4.4 | 0.2 | 4.0 | 1.1 | 2.6 | 0.6 | 3.7 | 0.5 | 2.1 | 0.6 | 2.9 | 0.6 | 3.7 | 0.7 |
| 22:0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.2 | 0.1 | 0.0 |
| 23:0 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.1 |
| 24:0 | 0.2 | 0.0 | 0.2 | 0.1 | 0.1 | 0.0 | 0.4 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.1 |
| ∑SFA | 43.1 | 0.4 | 46.3 | 0.6 | 48.3 | 3.3 | 44.8 | 2.3 | 46.6 | 2.9 | 45.5 | 1.5 | 47.6 | 2.3 |
| ∑MUFA | 31.9 | 0.6 | 28.9 | 2.2 | 29.8 | 1.1 | 28.2 | 1.5 | 31.4 | 2.0 | 29.6 | 2.0 | 28.2 | 1.0 |
| ∑PUFA | 25.0 | 0.8 | 24.8 | 2.4 | 21.9 | 3.6 | 27.0 | 3.3 | 22.0 | 3.6 | 24.9 | 3.0 | 24.2 | 2.8 |
| ∑n-3 | 14.9 | 0.1 | 14.8 | 1.7 | 11.3 | 2.4 | 14.0 | 2.0 | 9.8 | 2.1 | 12.4 | 1.9 | 13.4 | 2.4 |
| ∑n-6 | 9.9 | 0.7 | 9.9 | 0.9 | 10.5 | 1.6 | 12.8 | 1.9 | 11.8 | 1.5 | 12.3 | 1.6 | 10.5 | 1.1 |
| ∑Other FA | 4.8 | 0.1 | 4.3 | 0.2 | 3.7 | 0.2 | 4.5 | 0.2 | 4.4 | 0.3 | 4.0 | 0.4 | 4.1 | 0.2 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

**S10.5** Sire breed and sex interactions on *Longissimus dorsi* muscle tissue fatty acid composition [1]

| | Black Suffolk | | | | Dorset | | | | Merino | | White Suffolk (n35) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ewe | | Wether | | Ewe | | Wether | | Wether | | Ewe | | Wether | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 14:0 | 2.3 | 0.4 | 2.1 | 0.3 | 1.4 | 0.4 | 1.8 | 0.2 | 1.6 | 0.4 | 1.8 | 0.4 | 1.5 | 0.3 |
| 15:0 | 0.5 | 0.0 | 0.5 | 0.1 | 0.4 | 0.1 | 0.4 | 0.0 | 0.5 | 0.1 | 0.5 | 0.1 | 0.4 | 0.1 |
| 16:1ω9c | 0.3 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.2 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 |
| 16:1ω7c | 1.3 | 0.2 | 1.2 | 0.1 | 1.2 | 0.0 | 1.3 | 0.0 | 1.2 | 0.1 | 1.3 | 0.1 | 0.8 | 0.2 |
| 16:0 | 23.6 | 2.1 | 22.0 | 0.3 | 22.6 | 0.5 | 21.7 | 0.4 | 22.9 | 1.0 | 22.8 | 0.6 | 22.0 | 0.7 |
| 17:0 | 1.6 | 0.2 | 1.6 | 0.0 | 1.3 | 0.1 | 1.3 | 0.0 | 1.5 | 0.1 | 1.3 | 0.1 | 1.3 | 0.0 |
| 18:2ω6 | 4.0 | 0.4 | 3.4 | 0.1 | 3.3 | 0.5 | 4.3 | 0.6 | 5.0 | 0.5 | 4.5 | 0.8 | 3.6 | 0.4 |
| 18:3ω3 | 2.2 | 0.2 | 2.2 | 0.1 | 1.6 | 0.1 | 1.9 | 0.1 | 2.2 | 0.2 | 2.0 | 0.2 | 1.9 | 0.1 |
| 18:1ω9 | 32.0 | 4.2 | 38.6 | 0.4 | 36.7 | 1.2 | 36.6 | 0.6 | 33.6 | 0.9 | 35.3 | 1.2 | 37.6 | 1.4 |
| 18:1ω7c | 1.7 | 0.2 | 1.7 | 0.1 | 1.3 | 0.1 | 1.4 | 0.1 | 1.4 | 0.1 | 1.4 | 0.0 | 1.5 | 0.0 |
| 18:1ω7t | 3.6 | 0.3 | 3.1 | 0.1 | 2.7 | 0.1 | 2.5 | 0.1 | 3.0 | 0.2 | 2.7 | 0.2 | 3.1 | 0.2 |
| 18:0 | 19.8 | 0.8 | 18.6 | 1.0 | 21.6 | 1.1 | 19.8 | 0.7 | 20.7 | 0.6 | 18.0 | 1.1 | 19.8 | 0.9 |
| 20:4ω6 | 0.6 | 0.1 | 0.3 | 0.1 | 0.5 | 0.2 | 0.8 | 0.2 | 0.8 | 0.1 | 0.8 | 0.3 | 0.6 | 0.1 |
| 20:5ω3 | 0.8 | 0.1 | 0.2 | 0.2 | 0.3 | 0.1 | 0.6 | 0.1 | 0.4 | 0.1 | 0.5 | 0.1 | 0.3 | 0.1 |
| 20:3ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.2 | 0.1 | 0.1 | 0.0 |
| 20:4ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:2ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20:0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 |
| 22:5ω6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.1 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:6ω3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 | 0.1 | 0.0 |
| 22:5ω3 | 0.2 | 0.2 | 0.0 | 0.0 | 0.2 | 0.1 | 0.4 | 0.1 | 0.4 | 0.1 | 0.3 | 0.2 | 0.3 | 0.1 |
| 22:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 |
| 23:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ∑SFA | 48.7 | 3.5 | 45.5 | 0.3 | 48.1 | 1.7 | 45.8 | 1.0 | 47.9 | 0.6 | 45.4 | 1.5 | 45.9 | 1.7 |
| ∑MUFA | 43.1 | 3.6 | 48.1 | 0.2 | 45.3 | 1.2 | 45.4 | 0.8 | 42.6 | 0.9 | 45.7 | 1.8 | 46.7 | 1.3 |
| ∑PUFA | 8.2 | 0.2 | 6.3 | 0.5 | 6.6 | 1.0 | 8.8 | 1.2 | 9.5 | 0.7 | 8.9 | 1.5 | 7.4 | 0.8 |
| ∑n-3 | 3.2 | 0.1 | 2.4 | 0.3 | 2.3 | 0.3 | 3.1 | 0.4 | 3.2 | 0.4 | 3.1 | 0.4 | 2.7 | 0.3 |
| ∑n-6 | 4.7 | 0.3 | 3.7 | 0.2 | 4.1 | 0.7 | 5.5 | 0.8 | 6.1 | 0.4 | 5.6 | 1.1 | 4.4 | 0.6 |
| ∑Other FA | 5.4 | 0.4 | 4.3 | 0.4 | 4.1 | 0.2 | 4.3 | 0.2 | 3.9 | 0.3 | 5.8 | 1.3 | 4.5 | 0.5 |

[1] ∑SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA is the sum of 14:1ω5, 15:1ω6, 16:1ω9, 16:1ω7, Br17:1, 17:1ω8+a17:0, 17:1, 18:1ω9, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 22:1ω9, 22:1ω11, 22:1ω7, 24:1ω11, 24:1ω9, 24:1ω7; ∑PUFA is the sum of 16:3+16:4, 16:2, 18:4ω3, 18:3ω6, 18:2ω6, 18:3ω3, 20:4ω3, 20:4ω6, 20:5ω3, 20:3ω6, 20:2ω6, 21:5ω3, 22:6ω3, 22:5ω3, 22:5ω6, 22:4ω6, 24:6ω3, 24:5ω3;∑n-3 is the sum of 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:6ω3, 22:5ω3, 24:6ω3, 24:5ω3; ∑n-6 is the sum of 15:1ω6, 18:2ω6, 18:3ω6, 20:4ω6, 20:3ω6, 20:2ω6, 22:5ω6, 22:4ω6; ∑OtherFA is the sum of 14:1ω5, 15:1ω6, 16:3+16:4, 16:2, 16:1ω5, 16:1ω13, Br17:1, 17:1ω8+a17:0, 18:4ω3, 18:1, 20:2ω6, 20:1ω11, 20:1ω9, 20:1ω7, 20:1ω5, 21:5ω3, 21:0, 22:4ω6, 22:1ω9, 22:1ω11, 22:1ω7, 24:6ω3, 24:5ω3, 24:1ω11, 24:1ω9, 24:1ω7

*Appendix 4*

# Declarations

The following are declarations of co-authorship which describe B.W.B. Holman's contributions and involvements in chapter and paper formulation as per University of Tasmania, School of Agricultural Science / Tasmanian Institute of Agriculture PhD thesis requirements. Descriptions of chapter and paper status were provided within this thesis' List of Publications.

**Growth and body conformation responses of genetically divergent Australian sheep to *Spirulina* (Arthrospira platensis) supplementation**

*Co-authors*:

B.W.B. Holman, A. Kashani and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, data collection, data analysis and composition of paper.

*Signature of the co-authors*

**Effect of *Spirulina* supplementation on plasma metabolites in crossbred and purebred Australian Merino lambs**

*Co-authors*:

B.W.B. Holman and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, sample and data collection, sample and data analysis and composition of paper.

*Signature of the co-authors*

**Wool quality traits of grazing Australian purebred and crossbred Merino lambs orally drenched with *Spirulina* (Arthrospira platensis)**

*Co-authors*:

B.W.B. Holman, A. Kashani and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, sample and data collection, data analysis and composition of paper.

*Signature of the co-authors*

**Modelling the feed intake of purebred and crossbred Australian Merino lambs supplemented with *Spirulina***

*Co-authors*:

B.W.B. Holman and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
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| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, data collection, data analysis and composition of paper.

*Signature of the co-authors*

**Sire breed and sex variations in the fatty acid composition of heart, kidney, liver and muscle tissues of Australian lambs**

*Co-authors*:

B.W.B. Holman, A. Kashani, A.E.O. Malau-Aduli and P.D. Nichols

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, sample and data collection, sample and data analysis and composition of paper.

*Signature of the co-authors*

**Effect of nutritional plane on the fatty acid profiles of heart, kidney, liver, adipose and muscle tissues of Australian dual-purpose lambs**

*Co-authors*:

B.W.B. Holman, A. Kashani, A.E.O. Malau-Aduli and P.D. Nichols

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, sample and data collection, sample and data analysis and composition of paper.

*Signature of the co-authors*

**Effect of *Spirulina* supplementation and nutritional plane on Australian dual-purpose lamb liveweight, body conformation and growth**

*Co-authors*:

B.W.B. Holman, A. Kashani and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, data collection, data analysis and composition of paper.

*Signature of the co-authors*

**Effect of *Spirulina* (Arthrospira platensis) supplementation and plane of nutrition on wool quality from crossbred and purebred Merino lambs**

*Co-authors*:

B.W.B. Holman, A. Kashani and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: experimental design, field work, sample and data collection, data analysis and composition of paper.

*Signature of the co-authors*

**A review of sheep wool quality traits**

*Co-authors*:

B.W.B. Holman and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: design, literature analysis and composition of paper.

*Signature of the co-authors*

**_Spirulina_ as a livestock supplement and animal feed**

*Co-authors*:

B.W.B. Holman and A.E.O. Malau-Aduli

*Evaluation scale*:

1 – has contributed to this work (10-33%)
2 – has made substantial contribution to this work (34-66%)
3 – has made major contribution to this work (67-100%)

| | Declaration regarding specific elements | Extent (1, 2, 3) |
|---|---|---|
| 1 | Formulation/identification of the scientific problem that need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable via experiments | |
| 2 | Planning of the experiments and methodology design, including selection of methods and method development | |
| 3 | Involvement in experimental work | |
| 4 | Presentation, interpretation and discussion in a journal format of the obtained data | |
| Overall contribution | | |

*Explanation of student involvement in the work*:

Active involvement in: design literature analysis and composition of paper.

*Signature of the co-authors*